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The Administration of Di-Isopropyl Fluorophosphate (DFP) to Man. I. Effect on Plasma and Erythrocyte Cholinesterase; General Systemic Effects; Use in Study of Hepatic Function and Erythropoiesis; and Some Properties of Plasma Cholinesterase. D. Grob, J. L. Lilienthal, Jr., A. M. Harvey, and B. F. Jones 217

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THE ADMINISTRATION OF DI-ISOPROPYL FLUOROPHOSPHATE (DFP) TO MAN

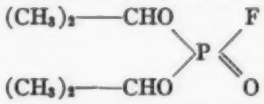
I. Effect on Plasma and Erythrocyte Cholinesterase; General Systemic Effects; Use in Study of Hepatic Function and Erythropoiesis; and Some Properties of Plasma Cholinesterase[1]

D. GROB, J. L. LILIENTHAL, Jr., A. M. HARVEY, and B. F. JONES

From the Physiological Division, Department of Medicine, The Johns Hopkins University and Hospital, Baltimore, Maryland

The alkyl esters of fluorophosphoric acid were discovered to be toxic inhalants by Lange and v. Krueger (23). Mackworth (27) demonstrated that the toxicity was due to the irreversible inhibition of cholinesterase (ChE) enzymes by these esters.

One member of this family of compounds has been subjected to particularly intensive study: *di-isopropyl fluorophosphate* (DFP).



Mazur and Bodansky (29) have demonstrated the extraordinary potency of DFP as an inhibitor of ChE both in vitro and in vivo and have shown in quantitative fashion that, in contrast to the transient effect of familiar anti-cholinesterase compounds, DFP inhibits irreversibly the ChE with which it comes in contact. The studies of Comroe (10), Modell (32), Horton (19), and their coworkers, and of Koelle and Gilman (21) indicated that the pharmacological effects of DFP administered to animals and man can be interpreted in terms of the ChE which had been destroyed.

The studies to be reported here and later record the observations made on certain effects of DFP noted during the course of its administration to more than 200 human subjects. Observations have been made on the following effects of DFP:

a. On the blood (plasma and erythrocyte ChE activity levels and regenerative characteristics),

[1] Work performed under a contract between the Medical Division, Chemical Corps, U. S. Army, and the Johns Hopkins University.

b. General systemic,
c. On the gastro-intestinal tract (14),
d. On the central nervous system (15),
e. On the peripheral neuromuscular unit (18).

The present communication reports the observations made on (a) and (b), above, in normal subjects, in patients with myasthenia gravis, and in patients with liver disease, severe anemia, acute febrile and chronic debilitating diseases, and renal disease.

METHOD OF DETERMINATION OF CHOLINESTERASE ACTIVITY

Cholinesterase activity of plasma and red blood cells was determined by the method of Ammon (2) as modified by Mazur and Bodansky (29), in which 0.2 cc.

TABLE 1

Plasma and red blood cell cholinesterase activity before the administration of DFP

SUBJECTS	NO.	AVERAGE VOLUME OF CO_2 EVOLVED (C.MM.)		RANGE (C.MM. CO_2)				STANDARD DEVIATION (C.MM. CO_2)	
		By plasma	By red blood cells	Plasma		Red blood cells		Plasma	RBC
				Low	High	Low	High		
Normal and convalescent...	75	138	130	100	209	80	211	25	18
Myasthenic.....	12	139	117	98	182	88	158	12	14

of plasma, or 0.5 cc. of a 25 per cent red blood cell solution in sodium bicarbonate, is allowed to hydrolyze a standard solution of acetylcholine (0.015 M) in a bicarbonate buffer (0.04 M) and the liberated carbon dioxide measured at 37° C. in a Warburg manometer for 30 minutes. ChE activity was expressed in c.mm. as the average of the volumes of carbon dioxide evolved during three consecutive periods of 10 minutes. The volume of carbon dioxide evolved by a control solution from which plasma and red blood cell solution were withheld was subtracted from the experimental determination.

THE CHOLINESTERASE ACTIVITIES OF NORMAL AND MYASTHENIC SUBJECTS

The plasma and red blood cell ChE activity of 75 normal and convalescent subjects, and of 12 myasthenic subjects were studied prior to the administration of DFP (Table 1). In confirmation of Lucas and his co-workers (24) and others no difference was observed in these two groups. The day to day variation in 12 myasthenic subjects was studied (each morning, prior to the administration of any medication),

and the average daily variation was found to be 7 c.mm. of carbon dioxide (5 per cent) for both plasma and red blood cells. Over a period of weeks the variation was greater, sometimes 10 per cent or more. Differences in ChE activity between individuals could not be correlated with age, sex, weight, menstrual cycle, exercise, diet, or moderate fasting, in agreement with the observations of Vahlquist (39) on non-myasthenic subjects.

THE EFFECT OF DFP ON PLASMA AND RED BLOOD CELL CHOLINESTERASE ACTIVITY

DFP was administered intramuscularly in 0.1 or 0.2 per cent solution in peanut oil, in which solvent the compound remains active for at least a year. When DFP was administered intravascularly an aqueous solution (1 mg. per cc.) was prepared immediately before injection. This was necessary as the aqueous solution is much less stable, its half hydrolysis time being about 16 hours (1). The effect of the drug on plasma and red blood cell ChE activity was studied in 35 subjects (25 normal and 10 myasthenic). The doses given and the resulting depression of ChE activity are summarised in Table 2.

A single dose of DFP administered either intramuscularly (0.5 to 3.0 mg.) or intra-arterially (0.5 to 2.0 mg.) caused a marked depression of plasma ChE to between 35 and 5 per cent of original activity, and a much less marked depression of red blood cell ChE to between 95 and 65 per cent of original activity (Figure 1). The maximum depression of plasma ChE, to near zero activity, occurred within one hour after administration of the drug, while red blood cell ChE activity declined more slowly, maximum depression occurring 24 hours after administration (Figure 2). The daily intramuscular administration of 0.5 to 2.3 mg. of DFP caused an immediate and sustained fall in plasma ChE to between 20 and 5 per cent of original activity, and a slower progressive decline of red blood cell ChE at an initial rate of 5 to 10 per cent per mg. of DFP per day (Table 2 and Figure 3).

THE RETURN OF CHOLINESTERASE ACTIVITY AFTER THE CESSATION OF DFP ADMINISTRATION

1. In Normal and Myasthenic Subjects

Following cessation of the administration of DFP the plasma ChE activity began to increase within four hours, and the red cell ChE activ-

TABLE 2

The effect of daily administration of DFP in oil on plasma and red blood cell cholinesterase activity

NUMBER OF SUBJECTS	AVERAGE DOSE DFP PER DAY		NUMBER OF DAYS OF ADMINISTRATION	TOTAL DFP		LOWEST CHOLINESTERASE REACHED*—% OF CONTROL	
	mg.	mg./kg.		mg.	mg./kg.	Plasma	RBC
Normal and Convalescent (25)							
1	0.5	0.010	1	0.5	0.010	35%	99%
2	1.0	0.014	1	1.0	0.014	18	90
1	0.75	0.013	2	1.5	0.027	19	81
1	2.0	0.033	1	2.0	0.033	18	90
1	3.0	0.054	1	3.0	0.054	14	71
2	1.0	0.016	3	3.0	0.048	13	66
1	1.5	0.020	3	4.5	0.060	6	52
1	1.0	0.016	5	5.0	0.082	11	48
2	1.1	0.018	5	5.5	0.092	8	45
6	2.0	0.029	3	6.0	0.077	11	38
1	2.3	0.030	3	7.0	0.090	10	44
1	1.3	0.028	6	8.0	0.168	12	20
2	1.3	0.023	7	9.0	0.158	4	24
2	1.4	0.026	7	10.0	0.170	1	25
1	1.3	0.022	8	10.5	0.174	4	23
Average.....	1.38	0.021	3.8	5.35	0.085	11.3	50.1
Myasthenic (10)							
1	0.42	0.013	11	4.6	0.141	10%	16%
1	1.00	0.015	10	10.0	0.144	10	42
1	0.46	0.005	27	12.4	0.185	13	41
1	1.69	0.023	8	13.5	0.185	9	46
1	0.44	0.009	37	16.2	0.316	9	23
1	1.25	0.025	25	31.2	0.623	10	22
1	1.04	0.019	33	34.3	0.636	10	22
1	0.66	0.013	56	37.0	0.840	8	48
1	1.13	0.020	33	37.2	0.667	6	15
1	0.69	0.012	64	44.0	0.734	7	3
Average.....	0.80	0.014	32.6	23.88	0.445	9.2	27.8

* 24 hours after the last dose of DFP.

ity within 24 to 48 hours and at a much slower rate (Figure 2). For plasma ChE the average rate of regeneration was 14 per cent of original activity during the first 24 hours, 9 per cent on the second day, 6 per

cent on the fifth day, 3 per cent on the eighth day and 2 per cent on the fifteenth day, while for red blood cell ChE the rate of regeneration was

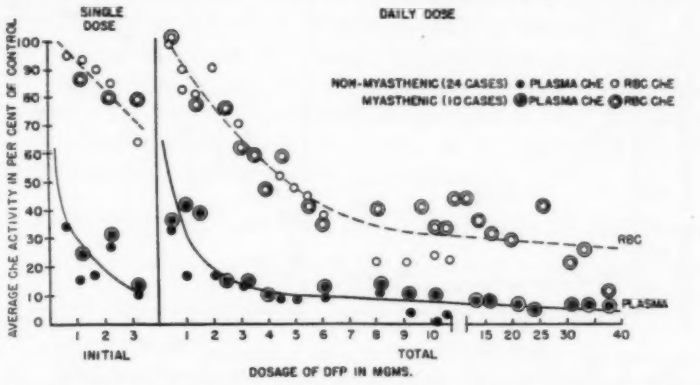


FIG. 1. THE EFFECT OF SINGLE AND DAILY INTRAMUSCULAR DOSES OF DFP IN OIL ON CHOLINESTERASE ACTIVITY (PLASMA AND RED BLOOD CELL)

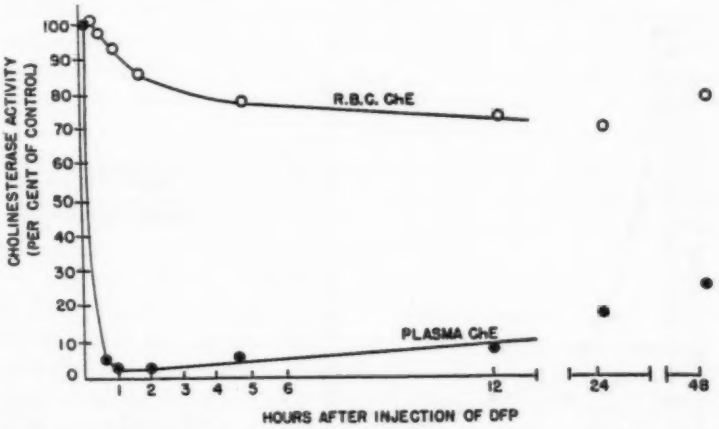


FIG. 2. THE EFFECT OF A SINGLE INTRAMSUCULAR INJECTION OF DFP IN OIL (2 MG.) ON CHOLINESTERASE ACTIVITY (PLASMA AND RED BLOOD CELL) (SUBJECT W. W.)

remarkably uniform at approximately one per cent of original activity per day (Figures 3 and 4).

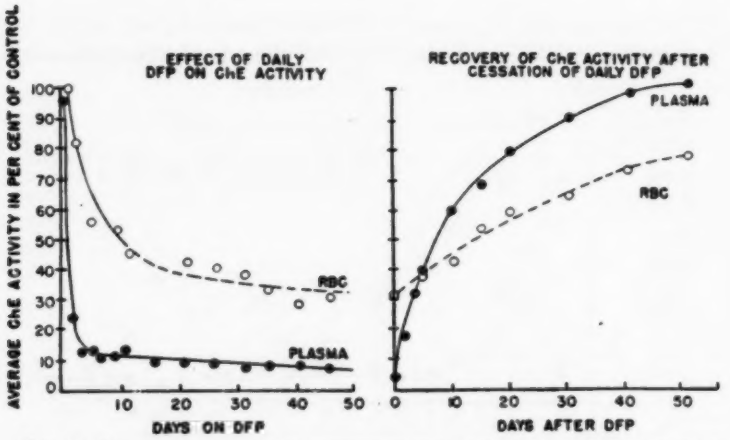

EFFECT OF DAILY DFP ON ChE ACTIVITY
RECOVERY OF ChE ACTIVITY AFTER CESSATION OF DAILY DFP
AVERAGE ChE ACTIVITY IN PER CENT OF CONTROL
RBC
PLASMA
DAYS ON DFP
DAYS AFTER DFP

FIG. 3. The effect of the daily intramuscular administration of DFP in oil on cholinesterase activity (plasma and red blood cell), and recovery after cessation of DFP. Average values obtained in 35 subjects (25 normal or convalescent, and 10 myasthenic).

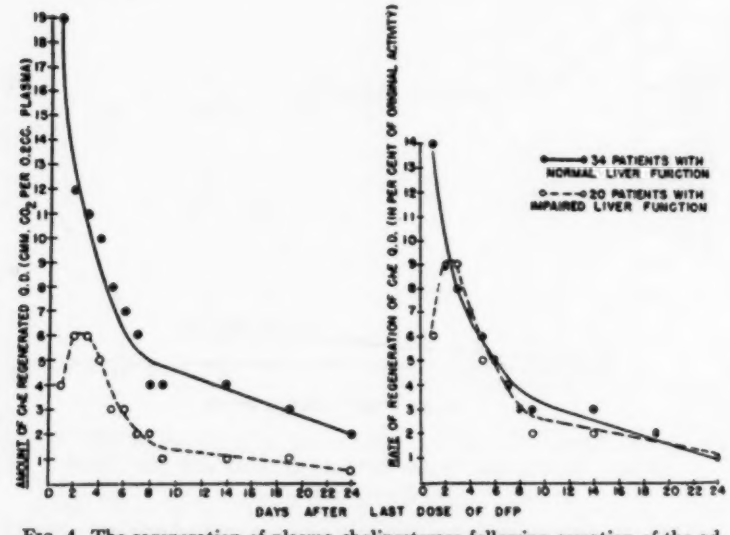

AMOUNT OF ChE REGENERATED O.D. (CMM. CO_2 PER 0.ECC. PLASMA)
RATE OF REGENERATION OF ChE O.D. (IN PER CENT OF ORIGINAL ACTIVITY)
34 PATIENTS WITH NORMAL LIVER FUNCTION
20 PATIENTS WITH IMPAIRED LIVER FUNCTION
DAYS AFTER LAST DOSE OF DFP

FIG. 4. The regeneration of plasma cholinesterase following cessation of the administration of DFP to normal subjects and to subjects with liver disease. (Average values represented.)

No subject who was followed for a sufficient length of time failed to show a return of the plasma and red blood cell ChE activity levels to those existing originally. Subjects who had previously received DFP and whose plasma and red blood cell ChE activity had returned completely to normal showed no evidence of sensitisation or of resistance to the drug. Finally, there was no significant difference between non-myasthenic and myasthenic subjects in the effect of DFP on plasma and red blood cell ChE activity, or on the rate of regeneration of ChE activity after the cessation of DFP administration.

2. Certain Regenerative and other Properties of Plasma Cholinesterase

Mazur and Bodansky (29) have presented strong evidence that DFP destroys ChE irreversibly. The restoration of ChE activity after the cessation of DFP administration, therefore, probably represents the regeneration of new enzyme protein. This relationship has provided an opportunity to study certain factors which govern the formation of ChE enzymes in man.

The rate of regeneration of plasma ChE in man after its depression by DFP was found to resemble the rate of regeneration of serum albumin in experimental animals which have been depleted by plasmaphoresis (20, 37). This indirect analogy suggested that a study of plasma ChE activity and regeneration in patients with disturbances of protein metabolism might provide information regarding the site of production and properties of this enzyme protein. Such studies in patients with hepatic disease, severe anemia, acute febrile and chronic debilitating diseases, renal disease, hemoconcentration, and serous transudates and exudates are described below.

a. *Hepatic disease.* The study of 80 patients with various disorders of the liver and of 75 normal and convalescent subjects revealed that 90 per cent of the patients with impaired liver function had a reduction in the level of plasma ChE activity below the normal minimum of 100 c.mm. of carbon dioxide. In general, the degree of depression of plasma ChE activity paralleled the severity of liver damage as reflected by a variety of hepatic function tests (Table 3, B–E). In contrast to the plasma ChE, the red blood cell ChE activity was not impaired. It is of some interest that the reduced plasma ChE activity in these patients was not accompanied by any recognizable cholinergic symptoms.

The depression of plasma ChE activity was not as great in the

TABLE 3

The influence of hepatic insufficiency, biliary obstruction, severe anemia, acute febrile and chronic debilitating diseases, nephrosis, and nephritis on plasma and red blood cell cholinesterase activity and regeneration. (*Average values recorded*)

	A	B	C	D	E	F	F[1]	G	H	I	J	K
	NORMAL CONTROLS	Non-obstructive hepatic insufficiency grouped in order of decreasing plasma ChE				Biliary obstruction		SEVERE ANEMIA	ACUTE FEBRILE DISEASES	CHRONIC DEBILITATING DISEASES	NEPHROTIC STAGE OF CHRONIC NEPHRITIS	ADVANCED CHRONIC NEPHRITIS
Total number of cases*	75	23	26	24	7	11	4	21	13	12	9	6
Plasma ChE activity (c.mm. CO_2)												
Range	100–209	84–110	56–83	29–55	14–28	105–135	31–82	57–129	24–125	36–130	114–243	93–113
Average	138	96	67	43	25	123	62	87	66	82	177	102
RBC ChE (c.mm. CO_2) average	130	146	137	141	136	139	122	116	124	129	129	118
Cephalin flocculation	0	1.5+	1.6+	2.1+	2.8+	0	0	0.6+	0.2+	1.0+		0
Thymol turbidity (units)	3	8	11	12	16	5.7	5.9	9.5	9.6	11		5
Prothrombin time (sec.) after vit. K admin.	16	19	22	24	31	17	18	19		19	16	17
Serum albumin (g.%)	4.0	3.7	3.3	2.9	2.5	4.1	3.8	4.0	3.4	3.7	1.7	4.1
Serum globulin (g.%)	2.8	2.9	3.3	3.4	3.4	2.5	2.8	2.6	2.5	3.5	2.1	2.5
Cholesterol (mg.%)	220	192	156	179	93	248	299	144	136	155	581	207
BSP retention (% after 30 min.)	5	21	30	32	44	24	44	14	9	11	3	5
Bilirubin (mg.%, total)	<0.8	3.2	4.6	10.0	21.4	9.0	12.6	2.4	1.1	1.1	<0.8	<0.8
Alkaline phosphatase activity (Bod. units)	4	5.6	6.2	10.8	6.3	17.7	23.7	4.1	3.1	5.1	3.9	2.9
	Amount of cholinesterase (c.mm. CO_2 per 0.2 cc. plasma) regenerated daily during week after administration of DFP (1.5 mg. I.M. q.d. for 3 days)											
Number of cases	35	7	7	6	0	3	3	5	3	8	3	2
Plasma	10	4.9	4.6	3.0		9.3	3.7	6.1	5.8	6.8	9.5	9.0
RBC	1.3	1.2	1.4	1.8		1.6	1.7	1.4			1.0	1.0

* Description of cases: A. Normal and convalescent subjects. B. Infectious hepatitis, 10; early cirrhosis, 7; metastatic carcinoma, 2; brucellosis, 1; lymphosarcoma, 1; Hodgkin's disease, 1; undiagnosed, 1. C. Cirrhosis, 20; leukemia, 1; lymphopathea venereum, 1; lymphosarcoma, 1; metastatic carcinoma, 1; undiagnosed, 2. D. Cirrhosis, 15; metastatic carcinoma, 5; sarcoid, 1; acute hepatic necrosis (terminal), 1; malaria, 1; undiagnosed, 1. E. Cirrhosis (terminal), 4; lymphoma (terminal), 3. F. Carcinoma, 5; biliary stone, 6. Average duration, 3.7 weeks. F[1]. Carcinoma, 3; biliary stone, 1. Average duration, 11.5 weeks. G. Post hemorrhagic, 4; pernicious anemia, 3; leukemia, 3; congenital hemolytic anemia, 2; sprue, 1; Banti's, 1; Felty's, 1; carcinomatosis, 1; thrombocytopenic purpura, 1; sickle cell anemia, 1; undiagnosed, 3. Average hematocrit, 22%. H. Pneumonia, 3; empyema, 2; tuberculous pleural effusion, 2; tuberculous pericarditis, 2; rheumatic fever, 2; gonococcal arthritis, 1; subacute bacterial endocarditis, 1. I. Lung abscess, 3; bronchiectasis, 2; lupus erythematosus, 2; pulmonary tuberculosis, 2; tuberculous peritonitis, 1; sarcoidosis, 1; eumatoid arthritis, 1. K. Average NPN, 110 mg.%.

TABLE 4

Changes in the plasma cholinesterase activity of patients with liver disease, anemia, and acute febrile illnesses. (Average values recorded)

	A. EFFECT OF PROGRESSION OF HEPATIC INSUFFICIENCY		B. EFFECT OF IMPROVEMENT OF HEPATIC FUNCTION		C. EFFECT OF RELIEF OF BILIARY OBSTRUCTION IN TWO PATIENTS WITH LOW PLASMA CHOLINESTERASE ACTIVITY		D. EFFECT OF RECOVERY FROM RELAPSE OF PERNICIOUS ANEMIA		E. EFFECT OF RECOVERY FROM ACUTE FEBRILE ILLNESSES	
Number of Cases	9		7		2		3		8	
Description	(3) Laennec's cirrhosis (2 terminal) (2) Lymphoma (terminal) (2) Lymphopathea venereum (1) Severe anemia (1) Metastatic carcinoma		(3) Infectious hepatitis (1) Cirrhosis (1) Pernicious anemia (1) Lymphopathea venereum (1) Malaria		(1) Carcinoma (1) Biliary stone		In relapse	After 3 wks. of folic acid or liver extract therapy	(3) Pneumonia (2) Empyema (2) Tbc pericarditis (1) Tbc pleural effusion	
							Av. hematocrit			
							16	35		
							Av. M.C.V.			
	On admission to hospital	3 wks. later	At height of illness	After improvement (6 wks. later)	During biliary obstruction	6 Weeks after relief of obstruction	137	112	During acute illness	2 weeks later
Cholinesterase Activity (c.mm. CO_2)										
Plasma range	40–84	21–45	64–93	103–179	31–40	50–57	64–100	103–130	24–74	84–125
Plasma average	60	30	70	131	35	54	73	120	53	108
RBC average	117	125	131	134	139	142	76	152	118	123
Cephalin flocculation	1.8+	2.5+	2.5+	0.2+	0	0	1.5+	0.5+	0.2+	0
Thymol turbidity	15	16	15	6	6	3	10	7.5	6	4
BSP retention (% after 30 min.)	35	45	24	10	40	11	11	7	9	4
Bilirubin (mg.%, total)	14.6	18.6	3.7	1.0	8.8	2.2	2.7	1.0	1.0	<0.8
Alkaline phosphatase activity (Bodansky units)	7	7	6	5	14	5	4	4	3	3

patients with infectious hepatitis as in those with other diseases of the liver having a comparable degree of damage as judged by the functional tests. This difference may perhaps be related to the shorter duration of hepatic disease in this group.

Progressive impairment of liver function was accompanied by a continued fall in plasma ChE activity, which reached very low levels in patients who died in hepatic insufficiency (Table 4, A). Conversely, those patients who exhibited evidence of improvement in liver function showed a slow increase in plasma ChE activity, which reached normal levels in patients who made a complete recovery (Table 4, B).

In addition, 15 patients with jaundice secondary to obstruction of the biliary tract were studied. As judged by the usual tests their hepatic function was unimpaired. Eleven of these patients had normal plasma ChE activity (Table 3, F), but four, who had had biliary obstruction of long duration, showed a significant reduction in the activity of their plasma ChE (Table 3, F[1]). In two of the latter group relief of biliary obstruction was effected, and with the waning of their jaundice the plasma ChE levels began to rise very slowly (Table 4, C).

The regeneration of plasma and red blood cell ChE was studied in the patients of each group (Table 3) by administering 1.5 mg. of DFP daily for three days and then measuring the daily rise in esterase during the following week. There was a rough parallelism between the degree of depression of plasma ChE existing before the administration of DFP and the amount of ChE regenerated after destruction by DFP (Table 3 and Figure 4). In addition, there was a direct relationship between the degree of hepatic damage and the regenerative capacity which was even more consistent than the relationship between the degree of hepatic damage and the plasma ChE activity before the administration of DFP. A distinction must be made between the *amount* of plasma ChE regenerated daily, which was considerably reduced, and the *rate* of regeneration of plasma ChE (expressed in per cent of activity before the administration of DFP), which was closer to normal. Neither the amount nor the rate of regeneration of red blood cell ChE was altered significantly in these patients.

These observations suggest that the reduction in plasma ChE activity in patients with hepatic damage reflects an impairment in the mechanism for production of this enzyme protein. The possibility exists

that this reduction in ChE activity may have resulted from increased inhibition or destruction of the enzyme, but there was no evidence to support this possibility. The incubation of plasma from patients with liver disease with normal plasma yielded a mixture whose ChE activity was the mean of those of the original plasmas. The dialysis of the plasma of five patients with liver disease against running tap water for 24 hours resulted in only a slight decrease in the ChE activity, from an average of 48 c.mm. of carbon dioxide to 39 c.mm., while the protein which was precipitated during the dialysis was found to possess no ChE activity when it was redissolved. These observations provide evidence against the presence of free or dialysable inhibitors of ChE in the plasma of patients with liver disease.

b. *Severe anemia, acute febrile and chronic debilitating diseases.* Depression of plasma ChE activity and of plasma ChE regeneration after the administration of DFP were not restricted to patients with outspoken diseases of the liver. Lesser, but significant, degrees of depression of both were encountered in patients with severe anemia (especially macrocytic anemia), and with a variety of acute febrile and chronic debilitating diseases (Table 3, G–I). The depression of plasma ChE regeneration after DFP in these patients was in general proportional to the existing depression of plasma ChE activity. There was no abnormality of the red blood cell ChE activity or regeneration in these patients, except for some cases of leukemia and of pernicious anemia in relapse, in which these too were depressed.

Many of these patients who had more marked depression of plasma ChE activity and regeneration also showed evidence of mild impairment of liver function, as reflected by the usual tests (Table 3, G–I). However, this was not invariable, and furthermore, the degree of reduction of both functions was usually more marked than the degree of liver impairment that was indicated by these tests. There was no observed relation of ChE activity to body temperature, bacterial invasion, leucocytosis, food intake, electrolyte balance, or inactivity. The addition of the plasma of these patients to normal plasma failed to demonstrate the presence of free inhibitors of ChE activity, and the dialysis of the plasma of these patients against running tap water for 24 hours failed to demonstrate the presence of dialysable inhibitors of ChE activity.

Recovery from the anemia or acute febrile illness resulted in a return

to normal of the plasma ChE activity (Table 4, D–E), and in diminution or disappearance of any signs of liver impairment. Improvement in chronic disease was accompanied by a more gradual increase in the plasma ChE activity.

c. *Renal disease.* Nine patients who were in the nephrotic stage of chronic glomerular nephritis with marked hypoalbuminemia and moderate hypoglobulinemia were found to have normal or elevated plasma ChE activity (Table 3, J). These patients, who had normal hepatic function by the ordinary tests, also had normal regeneration of plasma ChE after the administration of DFP. This is in contrast to the patients with liver disease, in whom reduction in the concentration of serum albumin was accompanied by reduction in the activity and regeneration of plasma ChE (Table 3, B–E).

TABLE 5

The effect of hemoconcentration due to acute mercury poisoning on cholinesterase activity, concentration of serum proteins, and hematocrit (9 cases, average values recorded)

TIME (HRS.) AFTER INGESTION OF BICHLORIDE OF MERCURY	CHOLINESTERASE ACTIVITY (C.MM. CO_2)		CONC. OF SERUM PROTEINS (G.%)			HEMATOCRIT
	Plasma	Red blood cells	Total proteins	Albumin	Globulin	
1	190	136	8.2	5.5	2.7	45
2	190	140	8.2	5.5	2.7	45
24	140	138	6.4	4.4	2.0	40
48	132	132	6.3	4.3	2.0	40

Six patients in the advanced stage of chronic glomerular nephritis had plasma ChE activities which are considered to be at the lower limits of normal. The regeneration of plasma ChE after its depression by DFP was approximately normal in two of the patients who were so studied. The red blood cell ChE activity and regeneration, the concentration of serum albumin and globulin, and the tests of liver function were normal in this group (Table 3, K).

d. *Hemoconcentration.* In patients with hemoconcentration, evidenced by an increase in plasma protein concentration and volume of packed erythrocytes, there was a proportional rise in plasma ChE activity (Table 5). There was no alteration in the ChE activity of the red blood cells. The hemoconcentration in the nine patients studied was

due to acute bichloride of mercury poisoning with loss of fluid and electrolytes into the gastrointestinal tract. All these patients received dimercaptopropanol (British Anti-Lewisite), but administration of this drug to seven normal individuals, resulted in no change in ChE activity, serum protein concentration, or volume of packed erythrocytes.

e. *Transudates, exudates, and urine.* The ChE activity of transudates, exudates, and urine was studied in 66 patients. Since addition of these fluids to normal plasma, or dialysis of the fluids, failed to demonstrate the presence of free or dialysable inhibitors of ChE, it has been assumed that the determined activity of the fluids was directly proportional to the amount of ChE which had diffused from the plasma. Contributions to the protein content of the fluids by the cells of the serous membranes and kidneys have, for the purpose of calculation, been assumed to be negligible.

The amount of ChE in transudates and exudates was the resultant of two functions: its level in the plasma and its diffusibility. Diffusion was in general proportional to, but slightly less than, the diffusion of serum albumin and globulin (Table 6). Transudates, and exudates with little protein, were practically devoid of ChE activity. Those exudates which contained larger amounts of protein had proportionately greater ChE activity. These relationships were independent of the membrane involved (peritoneal, pleural, pericardial, or synovial).

In marked contrast to the generally parallel diffusions of plasma proteins and ChE into exudates were the differences in their appearance in the urine of patients in the nephrotic stage of chronic glomerular nephritis, where the concentration of albumin usually exceeded that in the plasma, and the concentration of globulin approached that in the plasma, while the concentration of ChE never exceeded ten per cent of its plasma concentration (Table 6).

The urine of normal subjects was devoid of ChE activity. The urine of six patients with chronic nephritis, containing less than 0.3 g. per cent of protein, had little or no ChE activity (Table 6).

3. The Regenerative Properties of Red Blood Cell Cholinesterase

The average rate of regeneration of red blood cell ChE following its depression by DFP was found to be approximately 1.2 per cent of original activity per day (Figure 3). This figure is similar to the re-

placement rate of the red blood cells and indicates that the limiting factor in the regeneration of red blood cell ChE is the rate of replacement of the red blood cells themselves. There is some difference of

TABLE 6

The diffusion of albumin, globulin, and cholinesterase from the plasma into transudates, exudates, and urine

	DISEASE	BODY FLUID	NO. OF CASES	ALBUMIN CONCENTRATION* IN BODY FLUID (G. PER CENT)		GLOBULIN CONCENTRATION* IN BODY FLUID (G. PER CENT)		CHOLINESTERASE ACTIVITY OF BODY FLUID (C.MM. CO_2 PER 0.2 CC.)		PER CENT DIFFUSION FROM PLASMA INTO BODY FLUID conc. in body fluid / conc. in plasma × 100%		
				Range	Av.	Range	Av.	Range	Av.	Albumin	Globulin	ChE
Transudates	Nephrosis	Edema	3	0.01–0.02	0.01	0.01–0.02	0.02	0–1	0.3	<1	<1	<1
Exudates	Nephrosis	Pleural	1		0.1		0.1		1.5	9	5	1
	Cirrhosis	Ascitic	6	0.1 –0.4	0.3	0.1 –0.8	0.4	1–6	3.8	11	11	6
			1		2.6		1.5		16	67	53	20
		Pleural	1		0.8		0.6		7	30	17	9
	Neoplastic	Ascitic	2	0.6 –0.8	0.7	0.7 –1.1	0.9	13–15	14	24	26	21
		Pleural	1		1.5		1.3		12	38	50	19
	Polyserositis	Ascitic	2	0.6 –1.9	1.3	0.5 –1.0	0.8	13–38	26	33	33	29
		Pleural	5	1.3 –1.9	1.7	0.7 –1.9	1.4	13–56	41	52	52	42
	Cardiac failure	Ascitic	1		0.1		0.1		1	4	2	1
			3	1.4 –1.6	1.5	1.1 –1.3	1.2	18–30	26	41	60	43
		Pleural	1		1.4		1.3		14	52	35	48
	Infectious	Ascitic	2	1.3 –1.6	1.4	1.6 –2.0	1.8	15–18	17	55	47	39
		Pleural	1		0.4		0.2		7	17	7	12
			8	1.7 –3.7	2.7	0.8 –2.5	1.8	16–70	44	76	66	67
		Pericardial	4	1.9 –3.0	2.5	1.1 –4.1	2.3	24–60	42	70	74	70
		Joint	4	2.2 –3.8	3.0	0.9– 2.5	2.2	47–91	69	75	77	76
Urine	Normal	Urine	3	0	0	0	0	0	0	0	0	0
	Nephritis	Urine	6	0.03–0.2	0.1	0.02–0.1	0.04	0–2	0.5	2	2	<1
	Nephrosis	Urine	11	0.4 –4.0	2.3	0.2 –2.6	1.3	0–26	6	155	61	4

* Determined by fractional precipitation with neutral salt (Howe), except for the nephrotic urines, which were studied electrophoretically (Tiselius).

opinion regarding the normal replacement rate of circulating erythrocytes, but most of the experimental work (employing the method of selective agglutination of transfused erythrocytes and more recently radioactive and heavy isotopes) indicates a replacement rate of 0.8 to

1.2 per cent daily, corresponding to a life span for the red blood cell of 83 to 125 days (4, 7, 36, 40, 41). The average rate of regeneration of red blood cell ChE after its depression by DFP indicated a red blood cell replacement rate of 1.2 per cent daily, corresponding to a life span for the red blood cell of 83 days.

Additional evidence for the relationship between the regeneration rate of red blood cell ChE and the replacement rate of the red blood cells is furnished by a study of patients with altered red cell replacement rates. In patients with low reticulocyte counts there was a corre-

TABLE 7

The relation between the rate of regeneration of red blood cell cholinesterase after cessation of DFP and the reticulocyte count

NO. OF CASES	RETICULOCYTE COUNT (PER CENT)		DAILY RATE OF RISE OF ChE DURING FIRST WEEK AFTER CESSATION OF DFP (IN PER CENT OF CONTROL)			
			RBC ChE		Plasma ChE	
	Range	Average	Range	Average	Range	Average
13	0.2–1.0	0.7	0.2–1.9	0.8	5–13	7.1
24	1.1–2.0	1.4	0.7–1.7	1.2	6–11	7.6
6	2.1–3.0	2.6	2.3–2.9	2.6	5–12	7.6
1*		5.0		10.8		7.5
1†		10.8		13.0		9.5
1†		13.0		16.0		10.4
1†		13.6		17.0		10.5
1†		15.4		19.0		11.2

* Case M. D., pernicious anemia in relapse under liver therapy, during reticulocytosis.

† Case B. C., pernicious anemia in relapse under folic acid therapy, during reticulocytosis. Daily values given.

sponding low rate of regeneration of red blood cell ChE after cessation of DFP administration; and, conversely, in those patients with increased reticulocyte counts there was a corresponding increase in the regeneration rate of red blood cell ChE (Table 7). Furthermore, the rate of rise of red blood cell ChE activity in a given patient varied with the number of reticulocytes in the peripheral blood. For example, in one patient with pernicious anemia who was treated with folic acid the maximum reticulocyte response of 15 per cent was accompanied by a 19 fold increase in the rate of regeneration of red blood cell ChE (Table 7). In contrast, there was only a slight increase above normal in the

rate of regeneration of plasma ChE. Control studies have indicated that folic acid (in the absence of reticulocytosis) does not alter the plasma or red blood cell ChE activity either in vivo or in vitro.

In some cases of pernicious anemia in relapse and of leukemia the ChE activity of the red blood cells was below normal. The reticulocytosis which followed specific therapy of the former was accompanied by an increase in the ChE activity of the red blood cells. Thus, in three cases of pernicious anemia in relapse whose red blood cell ChE activity averaged 76 c.mm. of CO_2 before treatment, the daily administration of folic acid or liver extract resulted in a marked reticulocytosis and a sharp increase in the red cell ChE activity, which reached an average of 152 c.mm. of CO_4 after three weeks of therapy (Table 4, D). Similar observations have been reported by Sabine (34) following the administration of liver extract to patients with untreated pernicious anemia.

Davis (11) has suggested that the mechanism of the anti-anemic action of folic acid and liver extract is concerned with its influence on ChE activity. It is true that an increase in red blood cell ChE activity usually accompanied the reticulocytosis that followed the institution of therapy of pernicious anemia, but this rise in activity was found to be not essential to the anti-anemic effect of folic acid or liver extract. For, in two patients with pernicious anemia a complete remission was induced by folic acid and by liver extract therapy in spite of the sustained suppression of plasma and red blood cell esterase activity by the daily administration of DFP.

THE SYMPTOMS THAT FOLLOWED THE ADMINISTRATION OF DFP

The symptoms that followed the daily intramuscular administration to 60 subjects (50 normal and 10 myasthenic) of 1.5 to 3 mg. of DFP for two to five days mimicked most of the muscarinic and nicotinic effects of cholinergic drugs, with the exception of peripheral vasodilatation and of fall in blood pressure (Table 8). In addition, there were striking symptoms referable to the central nervous system.

In patients receiving small daily doses of DEP (1.5 mg. daily or less), central nervous system manifestations were usually the first to appear, followed by gastro-intestinal symptoms. The character of these central nervous system symptoms is illustrated in the case report below, while the frequency of their occurrence may be seen in Table 8. Their

TABLE 8

Symptoms following the daily intramuscular administration of DFP

EFFECTOR ORGAN	SYMPTOM	NO. OF SUBJECTS (TOTAL: 50 NORMAL AND 10 MYASTHENIC)
1. Muscarinic		
a) Gastro-intestinal		41
	1. anorexia-nausea	29
	2. abdominal cramps	25
	3. vomiting	14
	4. diarrhea	12
	5. cardiospasm	4
	6. nausea after smoking	4
b) Sweat glands	increased sweating	10
c) Lacrymal glands	increased lacrymation	3
d) Salivary glands	increased salivation	2
e) Pupils	slight miosis	2
f) Ciliary body	difficulty of distant vision	2
g) Lungs	respiratory difficulty, suggestive of bronchoconstriction	2
h) Bladder	urinary frequency	2
i) Heart	slight bradycardia with premature contractions	1
2. Nicotinic		14
Skeletal muscles	fasciculations (non-myasthenics only)	7
	increased strength (myasthenics only)	10
	decreased strength (non-myasthenics only)	4
	muscular cramps	1
3. Central nervous system		49
	excessive dreaming	33
	insomnia	29
	jitteriness, restlessness, increased tension, emotional lability, tremulousness	29
	nightmares, frequently with talking in sleep	17
	headache	8
	increased libido	6
	giddiness	6
	drowsiness	5
	paresthesias	3
	mental confusion	2
	visual hallucinations	1
	tremor	1
	pain in legs, sciatic distribution	1
4. Skin		
	urticaria (may have been sensitive to peanut oil)	2
5. No symptoms		6

intensity was proportional to the amount of DFP which the patients received. Accompanying electroencephalographic changes will be discussed in a subsequent report (15).

Those patients who received larger doses of DFP (more than 1.5 mg. daily) frequently developed gastro-intestinal symptoms first, followed by central nervous system symptoms. Anorexia was usually the first symptom to appear, followed by nausea, abdominal cramps, vomiting, and diarrhea as the degree of the DFP effect increased. Definite effects on other autonomic functions (sweating, lacrimation, salivation, miosis, ciliary spasm, bronchoconstriction, urinary frequency, and bradycardia) appeared much less frequently and usually only after larger doses of the drug.

The muscarine-like and central nervous system effects were the same in the normal and myasthenic subjects, while the nicotine-like effects were different. Muscular weakness and fasciculations occurred in some of the normal subjects after large doses of DFP, in contrast to the increased motor power, without fasciculations, that occurred in all the patients with myasthenia gravis.

The muscarinic and central nervous system symptoms disappeared within one to four days after the last dose of DFP. The nicotinic symptoms in non-myasthenic subjects disappeared within the same period. In myasthenic subjects who had received larger total doses of DFP (Table 2) the increased strength frequently persisted for as long as seven to ten days after the last dose of the drug.

The repeated administration of DFP resulted in an increasing effect of each dose, presumably because of the progressive irreversible inhibition of ChE. Repetition of the administration of DFP within three or four weeks of the last dose of the drug also resulted in an increased effect, presumably because the regeneration of ChE during this interval had not been complete.

Atropine given by any route had a marked inhibitory effect on the muscarinic symptoms due to DFP, a moderate inhibitory effect on the central nervous system symptoms, and no effect on the nicotinic symptoms. The administration of atropine was the best means of reducing the gastro-intestinal and central nervous system symptoms caused by DFP.

The following case report illustrates most of the manifestations that follow the administration of this drug.

A 56 year old white male subject was given daily intramuscular injections of DFP for three days. Twelve hours after the first injection of 3 mg. he began to have anorexia, mild nausea, and excessive dreaming. The content of the dream was that he was lost, deserted by his friends, and attacked by wild animals. On the following morning his plasma ChE was 16 per cent of normal activity and his red blood cell ChE 70 per cent. He was then given a second injection of DFP (2 mg.) and within two hours developed increased anorexia and nausea, restlessness, irritability, a feeling of tremulousness and tension, a dull headache and increased libido. That night he had insomnia and terrifying nightmares. The patient also had a nocturnal seminal emission, the first in many years. On the third day he was given another injection of 2 mg. of DFP. One hour later he developed marked anorexia, followed by nausea, vomiting, mild abdominal cramps, urgent defecation, sweating, and some generalised weakness. During the day he also had a sensation of tightness in the chest with a feeling of pressure on swallowing, suggestive of cardiospasm. The central nervous system symptoms became more marked, and he was also intermittently drowsy. That night the insomnia and nightmares were very troublesome, and the patient was heard to cry out frequently in his sleep. The following morning the plasma ChE was 10 per cent of original activity, and the red blood cell esterase 44 per cent. The symptoms diminished gradually and then disappeared during the next 48 hours.

THE RELATION OF SYMPTOMS DUE TO DFP TO THE CHOLINESTERASE ACTIVITY OF THE PLASMA AND RED BLOOD CELLS

Following the intramuscular administration of 1.5 mg. of DFP per day for five days, 2 mg. per day for four days, or 3 mg. per day for two days, the development of moderate symptoms was observed in almost all cases.

It became evident almost at once that the depression of plasma ChE activity bore no relation to the appearance of symptoms. A single dose of 1 to 2 mg. of DFP depressed the plasma ChE level almost to zero without the appearance of symptoms (Figure 2). There was, however, an apparent relationship between red blood cell ChE activity and the appearance of symptoms. When DFP was administered daily in doses of 1.5 to 3 mg. symptoms usually appeared when the red blood cell ChE level fell below 70 per cent of the original activity and became very troublesome when the level fell below 40 per cent. When, however, DFP was administered for long periods in smaller dosages (0.5 to 1.0 mg. each day), symptoms were absent or appeared transiently and then vanished although the red cell ChE activity fell below 25 per cent of original activity. Conversely, no matter how low the red blood cell

esterase had been depressed symptoms disappeared within 48 hours after DFP administration was stopped although the rise in red blood cell ChE activity during this time was negligible.

These observations indicate that the development of symptoms due to DFP bore no constant relation to the ChE activity of either the plasma or the red blood cells. The appearance of symptoms probably is related more directly to the activity level of ChE enzymes in the tissues of the effector organs themselves.

THE TOXICITY OF DFP

Ten patients with myasthenia gravis who received daily intramuscular injections of DFP for several weeks in total doses up to 0.84 mg. per kilo of body weight (Table 2) were studied by means of a number of examinations aimed at disclosing any general or specific toxicity other than the effects noted in Table 8. The administration of the drug produced no changes in pulse, temperature, respiratory rate, blood pressure, or body weight. No significant changes were noted in the number or distribution of leukocytes, number of erythrocytes, hemoglobin concentration, volume of packed red cells, number of platelets, renal function (urine analysis, phenolsulfonphthalein excretion and urea clearance), hepatic function (serum bilirubin, icteric index and bromsulfalein retention), chemical constituents of the blood (glucose, non-protein nitrogen, carbon dioxide combining power, cholesterol, calcium, phosphorus, total serum protein and albumin-globulin partition), electrocardiogram, teleroentgenogram of the chest, or basal metabolic rate. The stools did not contain occult blood even during the transient diarrhea that followed large doses of DFP and neostigimine.

Two of the patients who had received DFP daily for several weeks died 8 and 64 days after the last dose of the drug in an exacerbation of their myasthenia gravis which developed during acute infections of the respiratory tract. An autopsy was performed in each instance which disclosed purulent bronchitis and extensive areas of lobular pneumonia. There was hyperplasia of lymphatic tissue throughout the body in both cases, a lesion found not infrequently in myasthenia gravis. No other lesions were discovered on either gross or microscopic study.

DISCUSSION

1. The Regenerative and other Properties of Plasma Cholinesterase

Plasma ChE activity (which is identical with serum ChE activity) has been attributed to a mucoprotein which can be separated from the other plasma proteins and crystallised (5). Cohn and his coworkers (9) found that this enzyme was associated with their plasma protein fraction IV-6, 95 per cent of which consisted of alpha$_2$ globulin of molecular weight 300,000 and 5 per cent of albumin of molecular weight 69,000 (33). The results of the study of the movement of plasma ChE into serous exudates and urine has provided some indirect information regarding certain physical properties of this enzyme protein. The diffusions of plasma ChE, serum albumin, and serum globulin into serous exudates were roughly parallel, with the diffusion of ChE being in general slightly less than that of albumin and globulin. A similar relation for the diffusions of albumin, globulin and fibrinogen into serous exudates has been described (26). In contrast to their roughly parallel diffusions into serous exudates, the movements of plasma ChE, albumin, and globulin into the urine were markedly different, with albumin being lost into the urine in very large amounts, globulin to a moderate degree, and plasma ChE in only very small amounts. Again a similar relation for the appearance of albumin, globulin, and fibrinogen in nephrotic urine has been described (25). These observations indicate that of the several membrane barriers that were studied (peritoneal, pleural, pericardial, synovial, and renal) the nephrotic kidney was the only one that showed markedly different permeability to plasma cholinesterase, globulin, and albumin. In speaking of a renal membrane barrier it is understood that the concentration of a protein in urine is the resultant of at least two functions operating in opposite directions, glomerular filtration and tubular reabsorption. Diffusion across the renal barrier as calculated here, therefore, must represent the algebraic sum of the protein filtered through the glomerulus and that reabsorbed by the tubule. Since differences in the molecular weight of proteins are a factor influencing their diffusion across membranes (in addition to other factors such as equatorial diameter, charge, etc. (8)) the data suggest that the molecular weight of plasma ChE may be higher than that of the serum albumins (69,000)

and of most of the serum globulins (169,000) and may perhaps approach that of fibrinogen (400,000).

The ChE activity of the plasma has been studied in a variety of diseases, and has been reported to be reduced in liver disease (3, 30, 13), anemia (3, 13), acute infectious diseases (16), chronic debilitating diseases (31), cardiac failure, and renal disease (13). McArdle (30) proposed that the serum ChE activity be used an an index of liver function. Faber (13) observed that reduction of serum ChE activity was frequently accompanied by a parallel reduction in serum albumin concentration, and suggested that serum ChE and serum albumin may have a common site of origin, presumably the liver. These suggestions have been supported by the report of Brauer and Root (6) that the production of liver damage in rats by the administration of carbon tetrachloride resulted in a decrease in the plasma ChE activity, and have been refuted by Steensholdt and Venndt (38), who reported an increase in serum ChE activity following the production of liver damage in dogs by chloroform. Other experiments have indicated that this difference may be due to species differences in the cholinesterases of the liver and plasma (35), which make the results of ChE studies in some animals inapplicable to man. The observations reported in this paper add support to the hypothesis which emphasises the importance of the role played by the liver in the elaboration of plasma ChE. It will be noted that in disturbances of protein metabolism due to liver damage the basal level and regenerative capacity of plasma ChE were impaired in direct proportion to the hepatic insufficiency, while in disturbances of protein metabolism associated with renal disease the activity and regenerative capacity of plasma ChE were not impaired.

The deductions drawn above from the data on the restoration of plasma and red blood cell ChE activities following their depression by DFP, rest upon two assumptions. The first is that DFP destroys ChE irreversibly. Evidence for this has been furnished by the in vitro studies of Mazur and Bodansky (29). The second assumption is that DFP destroys the ChE within a brief period of time and that the drug itself is inactivated quickly in the body. Again Mazur (28) has provided evidence that there is available in the body tissues an enzyme, fluorophosphatase, which inactivates DFP rapidly. In view of this evidence it may be assumed reasonably that following the cessation of the administration of DFP no ChE will be released as a result of the

reversible activation of inactivated enzyme, and that there will be no destruction of regenerated ChE by DFP which remains active.

However, Mazur (28) has shown that the liver contains a higher concentration of fluorophosphatase than do other tissues, and it might be presumed that a relative insufficiency of this enzyme in patients with hepatic damage may account for the reduction in regenerative capacity observed in these patients. Inadequate levels of fluorophosphatase may perhaps explain the more striking reduction in plasma ChE regeneration observed in these patients during the first 24 hours after the administration of DFP was stopped (Figure 4), but it is an unlikely explanation for the over-all reduction in plasma ChE regeneration, since the regeneration of red blood cell esterase was not impaired. If the effect of DFP were prolonged by fluorophosphatase deficiency then one would anticipate a persistent depression in the red blood cell ChE activity as well. Furthermore, it is possible that the more marked reduction in plasma ChE regeneration observed in these patients during the first day after the cessation of DFP may be related not to fluorophosphatase deficiency, but to a diminished hepatic store of ChE, which is reported to occur in a damaged liver (6).

Outspoken liver disease was not the only cause of depression of plasma ChE activity and regeneration, since less marked, but significant, depression was also detected in anemias, and in acute febrile and chronic debilitating diseases. In these conditions some impairment of liver function was frequently detected, and is known to be common (30), but the degree of reduction of plasma ChE activity and regeneration was greater than the degree of liver impairment that was detectable by the ordinary tests. While it is possible that plasma ChE synthesis, presumably in the liver, may be more sensitive to inhibition by anemias, and by acute febrile and chronic debilitating diseases than are the usual tests of liver function, the possibility also exists that there may be other limiting factors than liver function which control the activity and synthesis of this enzyme protein. There was no evidence that free or dialysable inhibitors of ChE activity were an important factor in any of these conditions.

2. The Relation of Symptoms to the Cholinesterase Activity of Plasma and Red Blood Cells

It is very probable that the appearance and severity of cholinergic symptoms following the administration of DFP are related to the

depression of tissue cholinesterases in the effector organs themselves, and are unrelated to the ChE activity of either the plasma or the red blood cells. It is likely that the appearance of symptoms at a given level of red blood cell ChE activity following the administration of DFP for a short period of time is a coincidence, resulting from equal sensitivity of the cholinesterases in the tissues and in the red blood cells to DFP. This equality has been established in vitro by Mazur and Bodansky (29) for human erythrocytes and brain, in contrast to plasma ChE, which is much more sensitive to the action of DFP.

The lack of correlation between symptoms and red blood cell ChE activity following the administration of DFP in small doses over a longer period of time, or following the cessation of DFP, may be explained by two possible factors. If the regeneration of tissue cholinesterases following their depression by DFP were more rapid than that of red blood cell ChE (which is only 1.2 per cent per day) the tissue esterases might remain at a level compatible with normal function despite the progressive depression of the red blood cell ChE by small daily doses of DFP. Likewise, the tissue esterase activity might be restored appreciably within 48 hours after the cessation of DFP, thereby accounting for the disappearance of symptoms during this time, in which there is negligible regeneration of red blood cell esterase. In addition, there is the possibility that certain tissues may, through adaptation, function relatively normally at a level of tissue ChE considerably below the normal or optimal range.

The suggestion that tissue cholinesterases may be regenerated more rapidly than red blood cell ChE following their depression by DFP in human subjects is at variance with the observations of Mazur and Bodansky (29) and Koelle and Gilman (21) that in the rabbit and rat the red blood cell ChE is regenerated more rapidly. This difference between man and the rabbit or rat may perhaps be attributed to the more rapid replacement rate of red blood cells in the lower animals ($8\frac{1}{2}$ days (12, 17) in contrast to at least 80 days in man), resulting in the more rapid regeneration rate of red blood cell ChE.

3. The Toxicity of DFP

All of the symptoms and objective findings which were observed in patients who received DFP could be ascribed to the known effect of

this drug in inhibiting the action of ChE enzymes throughout the body, particularly in the autonomic and central nervous systems, and at the neuromuscular junction. There were no other toxic effects observed. However, great caution must be exercised in the use of this potent and potentially dangerous agent because of its cumulative action, and because of the long-lasting sensitisation[2] that follows its administration. Furthermore, the administration of lethal doses of DFP to animals (32, 22) has resulted in fatal circulatory and respiratory depression without demonstrable changes in the organs and tissues at autopsy.

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SUMMARY

1. The administration of DFP to human subjects caused an immediate marked depression of plasma cholinesterase activity and a much slower depression of red blood cell cholinesterase activity.

2. The cholinesterase activity of the plasma, and its regeneration following depression by DFP, were reduced in patients with liver disease in proportion to the degree of hepatic insufficiency.

3. Plasma cholinesterase activity and regeneration were also reduced in severe anemia, and in acute febrile and chronic debilitating diseases. Plasma cholinesterase activity was normal or elevated in nephrosis, and at the lower limits of normal in advanced chronic nephritis.

4. The diffusions of plasma cholinesterase, serum albumin, and serum globulin into serous exudates were roughly parallel. In contrast, their movements through the nephrotic kidney were markedly different, with albumin appearing in the urine in very large amounts,

[2] "Sensitisation", in this series of papers, connotes simply an apparent lowering of threshold and does not imply the existence of an allergic phenomenon.

globulin in moderate amounts, and cholinesterase in much smaller quantities.

5. The regeneration of red blood cell cholinesterase following its depression by DFP proceeded at a rate determined by the replacement rate of the red blood cells themselves (1.2 per cent daily). This rate, which was proportional to the number of circulating reticulocytes, became extremely high in patients with pernicious anemia during the early phases of specific therapy.

6. The administration of DFP caused symptoms which duplicated the muscarinic and nicotinic effects of cholinergic drugs in general, and, in addition, symptoms referable to the central nervous system. The administration of atropine inhibited the muscarine-like and central nervous system symptoms, but had no effect on the nicotine-like symptoms.

7. The symptoms caused by DFP are explicable in terms of the inhibition of the cholinesterase enzymes of the tissues themselves, and are not related to the cholinesterase activity of the plasma or red blood cells.

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THE ADMINISTRATION OF DI-ISOPROPYL FLUOROPHOSPHATE (DFP) TO MAN

II. Effect on Intestinal Motility and Use in the Treatment of Abdominal Distention[1]

D. GROB, J. L. LILIENTHAL, JR., AND A. M. HARVEY

From the Physiological Division, Department of Medicine, Johns Hopkins University and Hospital, Baltimore, Maryland

Di-isopropyl fluorophosphate (DFP) is a potent anticholinesterase agent which permanently inactivates cholinesterase (ChE) with which it comes in contact (6). The general systemic effects of DFP, and its effect on plasma and red blood cell ChE activity have been presented in a previous report (2). Following the daily intramuscular administration of DFP to 60 normal subjects gastrointestinal symptoms usually occurred, including, in order of frequency, anorexia, nausea, abdominal cramps, vomiting, diarrhea, and symptoms of cardiospasm. It was observed that these symptoms were increased by the administration of neostigmine or pitressin in doses that had been ineffective before the administration of DFP, and were suppressed by atropine. The administration of neostigmine shortly before DFP was observed to diminish somewhat the symptoms produced by the latter.

THE EFFECT OF DFP ON INTESTINAL ACTIVITY

Intestinal activity was recorded in 26 experiments on three subjects by means of a water manometer-air tambour recorder which was connected to a water-filled Miller-Abbott balloon lying in the intestine. Such a recorder does not measure absolute intralumenal pressure with accuracy (1) but does record changes in intestinal motility and tone.

The intramuscular injection of 1 to 3 mg. of DFP in 0.1 per cent solution in peanut oil caused a marked increase in the motility of the small and large intestine, with rhythmic contractions of greatly increased amplitude beginning about one hour after injection of the drug and occurring at the rate of one every two or three minutes over a

[1] Work performed under a contract between the Medical Division, Chemical Corps, U. S. Army, and the Johns Hopkins University.

period of two to five hours (Figure 1). The frequency of the contractions was greatest initially and decreased with time. Occasionally, relaxation following a muscular contraction was slow, with the contraction persisting for several minutes, but, in general, the resting tone of the intestine was not changed during the period of augmented activity. During this period of increased intestinal activity numerous borborygmi were audible over the abdomen, and with the larger dose of 3 mg. nausea and abdominal cramps sometimes appeared.

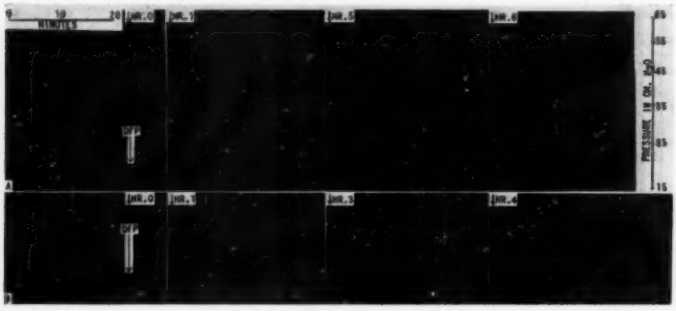

FIG. 1. THE EFFECT OF DFP ON THE MOTILITY OF THE ILEUM AND COLON
A. DFP, 1 mg. in oil, administered intramuscularly to subject T. D. (ileostomy). Rhythmic contractions of the ileum began one hour later and persisted for five hours. *B*. DFP, 2 mg. in oil, administered intramuscularly to subject G. J. (cofostomy). Rhythmic contractions of the colon began one hour later and persisted or three hours.

INHIBITION OF THE DFP EFFECT

The increased intestinal motility due to DFP could be inhibited temporarily by the administration of atropine, morphine, or demerol (isonipecaine, meperidine) (Figure 2). Atropine and demerol, which decreased the tone and motility of the normal small intestine, had a similar effect during the activity induced by DFP. Morphine, which increased the tone and decreased to some extent the motility of the normal small intestine, likewise had a similar effect after the administration of DFP. The diminution of motility was greater following atropine or demerol than after morphine.

POTENTIATION OF THE ACTION OF OTHER DRUGS BY DFP

Since DFP inhibits cholinesterase irreversibly, it would be expected to exert an effect until the enzyme has regenerated to a level compatible

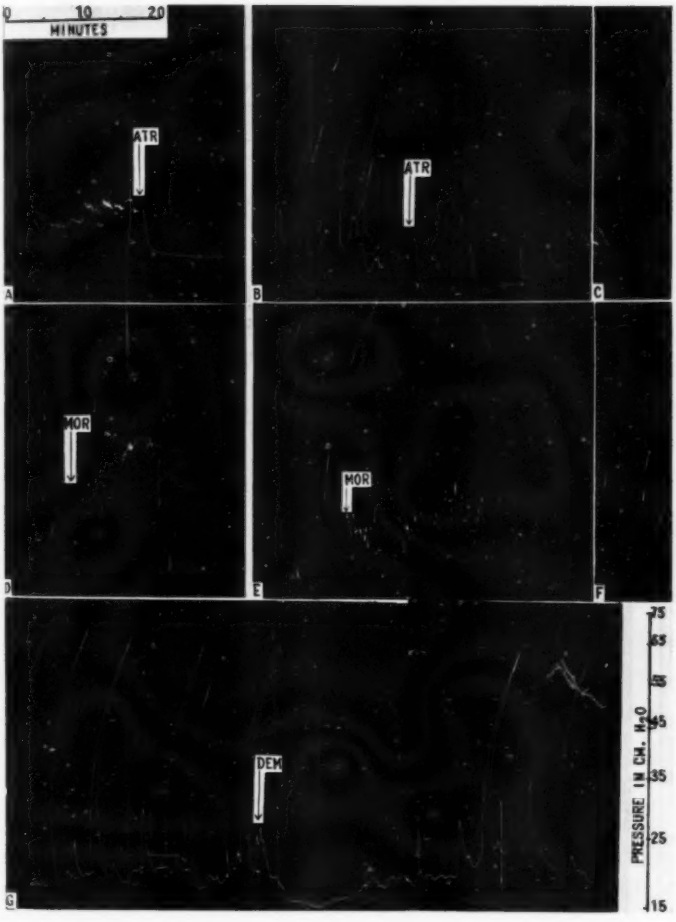

FIG. 2. THE INHIBITION BY ATROPINE, MORPHINE, AND DEMEROL OF THE INCREASED INTESTINAL MOTILITY DUE TO DFP
(SUBJECT T. D., ILEOSTOMY)

A and D. The effect of atropine (1.2 mg. intravenously) and morphine (12 mg. intravenously) on the normal intestine. The effect of demerol (50 mg. intravenously) was the same as that of atropine. *B, E, and G.* The effect of atropine, morphine, and demerol (in the above doses) on the increased intestinal motility due to DFP (2 mg. administered intramuscularly one hour (*B*), 1½ hours (*D*), and two hours (*G*) previously). *C, F, and G.* The return of increased intestinal motility 80 minutes (*C*), 87 minutes (*F*), and 30 minutes (*G*) later.

with normal function. Twenty-four hours after the last dose of DFP the intestinal motility was still slightly increased, as manifested by the increased frequency and amplitude of the small rhythmic waves. (Figure 3B). In addition, the intestine was found to be sensitised to

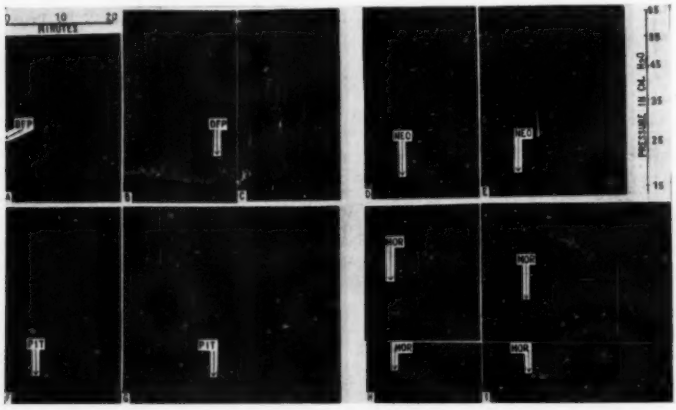

FIG. 3. THE POTENTIATION BY DFP OF THE ACTION OF DFP, NEOSTIGMINE PITRESSIN, AND MORPHINE ON THE ILEUM. (SUBJECT T. D., ILEOSTOMY)

A, DFP, 0.1 mg. I.M.; *D*, neostigmine, 0.05 mg. I.V.; *F*, pitressin, 1 unit I.V.; *H* (lower), morphine, 3 mg. I.V.: Subeffective doses, administered before the patient received DFP. *B*, DFP, 0.1 mg. I.M.; *E*, neostigmine, 0.05 mg. I.V.; *G*, pitressin, 1 unit I.V.; *I* (lower), morphine, 3 mg. I.V.: The same doses administered 24 to 40 hours after the last dose of DFP (2 mg. q.d. for 2 days), at which time ChE activity was approximately 5 per cent (plasma) and 50 per cent (red blood cells) of control. DFP, neostigmine and pitressin now cause increased intestinal motility and cramps, while the morphine now causes increased intestinal tone. *H* (upper), morphine 12 mg. I.V. administered before the patient received DFP. *I* (upper), morphine 12 mg. I.V. administered 48 hours after the last dose of DFP.

the action of DFP, neostigmine, pitressin, morphine and inhaled tobacco smoke (Figures 3 and 4). The potentiation of the action of DFP, neostigmine and pitressin was most striking. Doses of these drugs which had produced no effect before the administration of DFP now caused intestinal activity sufficient to produce nausea and abdominal cramps after sensitisation of the intestine by DFP. The potentiation

of the action of neostigmine was also demonstrable when injected during augmented intestinal activity due to DFP. A previously ineffective dose of neostigmine resulted in increased intestinal activity with the development of abdominal cramps (Figure 5).

FIG. 4. THE POTENTIATION BY DFP OF THE EFFECT OF SMOKING ON THE ILEUM (SUBJECT T. D., ILEOSTOMY)

A. Slight effect of smoking three cigarettes before the subject received DFP. *B*. Increased intestinal motility and cramps following the smoking of three cigarettes 24 hours after the last dose of DFP (2 mg. q.d. for two days).

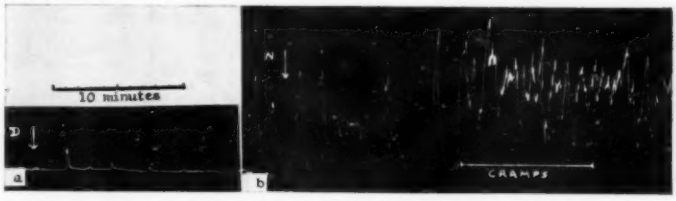

FIG. 5. THE POTENTIATION OF THE ACTION OF NEOSTIGMINE ON THE JEJUNUM BY DFP DURING THE TIME OF INCREASED MOTILITY DUE TO DFP (SUBJECT W. W.)

A. DFP, 2 mg. in oil, intramuscularly. *B*. Rhythmic contractions, which began one hour after (*a*), recorded two hours after administration of DFP. A previously ineffective dose of neostigmine (1 mg. intramuscularly) now causes increased motility and cramps.

The duration of this increased sensitivity of the intestine to stimulating drugs was investigated in 15 subjects by the daily administration of graded doses of neostigmine and pitressin before, during, and after the period of injection of DFP, with determination of the intramuscular dose required to produce abdominal cramps. Doses of neostigmine and of pitressin which produced no symptoms previously caused abdominal cramps, nausea, and frequently vomiting and diarrhea when

given during, or for a period of one to three weeks after, the administration of DFP (Figure 6).

THE TREATMENT OF ABDOMINAL DISTENSION WITH DFP

The prolonged effect of DFP on intestinal motility and its potentiation of the effects of neostigmine and pitressin suggested a therapeutic trial of this drug in the treatment of abdominal distension. Sixty-four

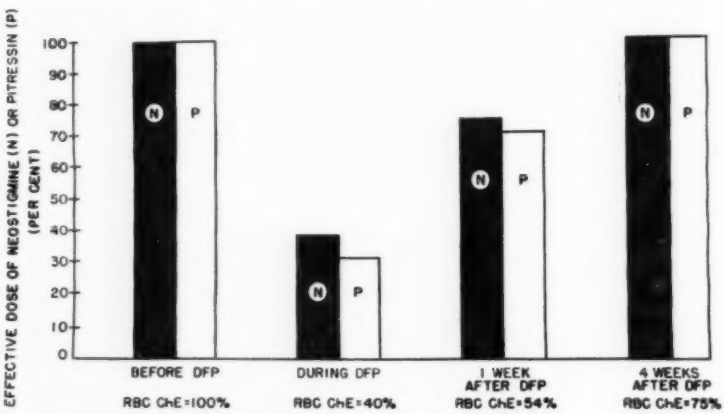

FIG. 6. The increased sensitivity of the gastrointestinal tract to intramuscular neostigmine and pitressin during and after the daily intramuscular administration of DFP to 11 subjects (eight normal and three myasthenic). The effective dose is that required to produce abdominal cramps. Each block represents the average of the 11 subjects. The results were similar in normal and myasthenic subjects except that the latter were less sensitive to neostigmine than the former.

patients with moderate to severe paralytic ileus were treated with intramuscular DFP, supplemented in 46 of the more severe cases by the hypodermic injection of neostigmine. Forty-six of these patients had distension following major abdominal operations, eight had peritonitis (tuberculous (2), post-operative (2), gonococcal (1), and due to perforating wound of the abdomen (3)), five had severe pneumonia, and five had lesions of the spinal cord (due to transverse myelitis, syringomyelia, epidural abscess, ruptured intervertebral disc, and vertebral

fracture). The distension in these patients had not responded to enemas and the use of a rectal tube. The 46 more severe cases (28 of the post-operative cases and all the remainder) had also been given 0.5 to 1.0 mg. neostigmine (supplemented in 25 cases by 10 to 20 units of pitressin) every two hours without adequate effect.

In the less severe cases relief was obtained from the intramuscular administration of DFP alone. One to two hours after the initial dose of 2 mg. of DFP there was evidence of increased intestinal motility, manifested by borborygmi, the passage of flatus, decreased nausea, and some relief of the distension. This persisted for from two to eight hours. The subsequent administration of DFP (at 8 to 24 hour intervals) resulted in evidence of increasing intestinal motility, with the frequent appearance of mild cramps, spontaneous defecation, and subsidence of the distension. The effect of the DFP (in peanut oil) was much more prolonged than that of neostigmine or pitressin (in aqueous solution), and was rarely, in the doses employed, accompanied by troublesome abdominal cramps.

In the more severe cases, whose distension was not fully relieved by DFP alone, good results were obtained by the additional use of neostigmine in doses which had previously been ineffectual. The administration of 0.5 to 1 mg. of this drug was followed by the passage of flatus and frequently by a bowel movement, with marked lessening of the distension. The administration of pitressin (10 to 20 units) produced a similar effect, and the combined use of the two drugs following sensitisation of the intestine by DFP produced the best results.

The optimal dosage of DFP, and the best combination with other drugs, has not yet been fully determined. The following schedule has produced satisfactory results within 4 to 24 hours after the onset of therapy:

An initial dose of 2 mg. of DFP was administered, followed in three and seven hours by neostigmine (0.5 to 1 mg. hypodermically) and pitressin (10 to 20 units hypodermically). One mg. of DFP was then administered at 12 hour intervals for two or three doses, with neostigmine and pitressin three and sometimes seven hours after each injection. One mg. of DFP was injected on subsequent days if necessary to prevent or treat the recurrence of distension, and neostigmine and pitressin were added as needed.

In order to obtain the maximum effect of DFP in the treatment of abdominal distension, it proved desirable to withhold drugs which suppress the increased intestinal motility that follows the administration of DFP. Thus, atropine and demerol were not given, unless necessary to control symptoms of DFP overdose. The administration of morphine to control pain was timed when possible so as not to coincide with the period of maximum action of DFP. Finally, in view of the observation that the administration of neostigmine shortly before DFP diminished the anticipated symptoms of the latter, DFP was not administered until three or more hours after the last dose of neostigmine. On the other hand, neostigmine was given at any time after the action of DFP had begun, and usually was a necessary adjunct in the treatment of moderate or severe distension.

The potent actions of DFP made necessary careful regulation of dosage and close watch for the development of central nervous system symptoms. Of the 64 patients treated with DFP for abdominal distension in the doses described above, the only untoward symptoms that were encountered were excessive dreaming in eight patients, nightmares in three, vertigo in one, paresthesias in one, and increased delirium in one patient severely ill with pneumonia. These symptoms all disappeared within 48 hours after the cessation of DFP.

In order to avoid the unpleasant and potentially dangerous side effects of DFP the amount of DFP administered on the first day should not exceed 3 or 4 mg., and on subsequent days 1 or 2 mg. In most instances the drug should be discontinued after one week of daily administration, or a total dose of 10 mg. Should further administration be desired the daily dose should be reduced below 1 mg., and the patient followed with extreme care, preferably with the help of serial determinations of the red blood cell ChE activity. Since the effective intramuscular dose of DFP is related to body weight, the dosage should be less in small or emaciated patients.

It must be emphasized that under no circumstances should this drug be given to a patient until the possibility of an organic obstruction of the gastrointestinal tract has been excluded. If vomiting or abdominal pain occurs due to DFP this can be readily inhibited by the administration of atropine.

DISCUSSION

Mode of Action of DFP

The increased intestinal motility that follows the administration of DFP is believed to be due to the irreversible inhibition by DFP of part of the ChE localised in the intestine. Measurement of the local concentration of ChE was not possible, but the time relation of the in-

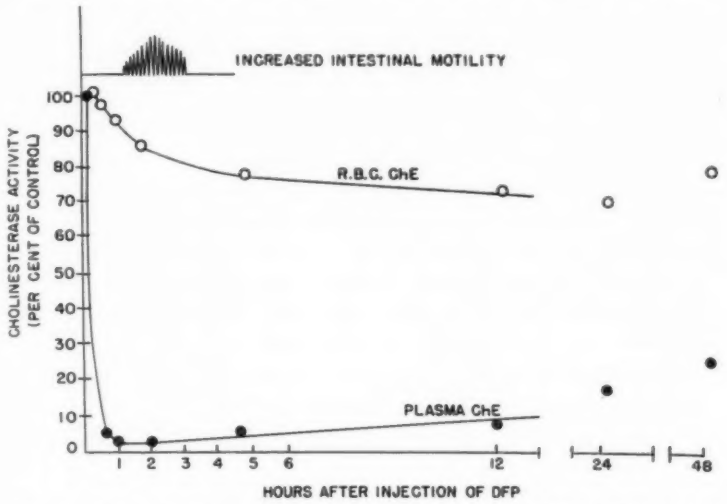

FIG. 7. The effect of a single intramuscular injection of DFP in oil (2 mg.) on cholinesterase activity (plasma and red blood cell) and intestinal motility. (Subject W. W.). The upper line represents schematically the appearance of intestinal hyperactivity.

creased intestinal motility to the concomitant depression of plasma and red blood cell ChE was determined (Figure 7). Evidence obtained from the study of experimental animals (5), and the correlation in human subjects between red blood cell ChE activity and the onset of symptoms following a brief period of administration of DFP (2), suggest that following a single dose of DFP the reduction of tissue ChE concentration probably is indicated better by the fall in red blood cell rather than plasma activity.

The subsidence of intestinal overactivity within three to six hours after the administration of DFP cannot be due to local regeneration of ChE to normal levels, for this has been shown to require many days (6). This disappearance of spontaneous overactivity may perhaps represent a process of local adaptation with a rapid initial regeneration of tissue ChE to a level where acetylcholine is hydrolysed at relatively normal rates. The slow disappearance of hypersensitivity of the gastrointestinal tract during the one to three weeks after DFP administration probably does reflect the local regeneration of ChE to normal levels, and may be compared with a similar process occurring in relation to neuromuscular function and electrical activity of the brain after cessation of DFP (3, 4).

The observation that the administration of neostigmine shortly before DFP diminished the anticipated symptoms of the latter parallels a similar phenomenon observed in the study of neuromuscular function (4) and illustrates a difference in the mode of action of these drugs. It is believed that neostigmine, in contrast to DFP, combines reversibly with ChE, and that the ChE so bound is not susceptible of destruction by DFP. On the other hand when neostigmine is administered after DFP has exerted its effect on the intestinal ChE (Figures 3E and 5) further increase in intestinal activity is produced, presumably because of the inhibition by neostigmine of the remaining local ChE that has escaped destruction by DFP.

The observation that DFP potentiates the action of pitressin and of morphine on the intestinal tract is of considerable physiological interest, since the latter drugs are said to exert their effect by direct action on smooth muscle itself in contrast to the anticholinesterase compounds, DFP and neostigmine, which are thought to act primarily at the nerve-ending.

Necessary Precautions in the Use of DFP

It must be emphasised that DFP exerts a cumulative effect which is very slowly reversible, and that in overdose in experimental animals death follows the development of respiratory and circulatory failure, probably central in origin (7). In man it has been observed invariably that repeated doses of DFP exert an increasing effect, presumably because of the progressive irreversible inhibition of ChE. Similarly,

since ChE is slowly regenerated after the cessation of DFP the sensitisation produced by this drug is long lasting, so that should further administration of DFP be desirable within three or four weeks after the last injection of the drug, the dose must be reduced. Following repeated administration of DFP the red blood cell ChE activity gives only a very approximate indication of the state of the tissue ChE as it is apparently more slowly regenerated. The plasma ChE activity probably bears no relation at all to symptoms or to sensitisation. Therefore, these determinations are of limited practical use in regulating dosage (2).

DFP has not caused peripheral vasodilatation or fall in blood pressure, even in the relatively large dose of 3 mg. a day for three days. It has caused bradycardia (with premature contractions) in only one case. Clinical observations to date have not indicated that the supplementary use of neostigmine in moderate doses results in a dangerous decrease of blood pressure or in marked bradycardia. However, animal experiments have demonstrated that hypotension following acetylcholine is considerably potentiated by the previous administration of DFP (7). This suggests that, unless further investigations indicate otherwise, acetylcholine and other direct cholinergic agents should be withheld from patients receiving DFP.

The occurrence of increased delirium following the administration of DFP to one severely ill patient may or may not be attributable to the drug, but indicates the advisability of cautious administration of DFP to severely ill patients. Similarly, although DFP has been administered to two patients with a history of asthma without untoward results the occurrence of bronchoconstriction following the injection of large doses of DFP in experimental animals (6) indicates that caution should be exercised when the drug is used in patients with a history of allergic disease.

SUMMARY

(1) The administration of DFP, a potent anticholinesterase drug, to human subjects caused a marked increase in the motility of the small and large intestine, which lasted, after a single injection, for two to five hours.

(2) The increased motility due to DFP was inhibited temporarily

by atropine, morphine, and demerol. It was increased by neostigmine.

(3) The administration of DFP sensitised the intestine to the action of DFP, neostigmine, pitressin, morphine, and inhaled tobacco smoke. This sensitisation lasted for one to three weeks and is believed to be due to the irreversible inactivation by DFP of intestinal cholinesterase, which then is regenerated slowly.

(4) Sixty-four patients with abdominal distension were treated successfully with DFP, supplemented in most cases by neostigmine. The best results were obtained by administering neostigmine and pitressin after sensitisation of the intestine by DFP.

(5) The central nervous system symptoms that may be caused by DFP were not prominent with the doses of the drug that were employed. However, because the repeated administration of DFP results in a cumulative irreversible inhibition of cholinesterase, and thus has potentially dangerous effects, caution should be exercised in its use.

Acknowledgement: We are indebted to Dr. Myron I. Buchman of the Gynecological Service for his assistance in treating many of the cases of abdominal distension, and to Dr. John H. Brewer of Hynson, Westcott, and Dunning for the ampouling of the DFP solutions that were employed.

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THE ADMINISTRATION OF DI-ISOPROPYL FLUOROPHOSPHATE (DFP) TO MAN

III. Effect on the Central Nervous System with Special Reference to the Electrical Activity of the Brain[1]

D. GROB, A. M. HARVEY, O. R. LANGWORTHY AND J. L. LILIENTHAL, JR.

From the Physiological Division, Department of Medicine, and the Department of Psychiatry, Johns Hopkins University and Hospital, Baltimore, Maryland

The general systemic effects of the potent anticholinesterase compound, di-isopropyl fluorophosphate (DFP), and its effects on plasma and red blood cell cholinesterase activity and on intestinal motility have been presented in previous reports (6, 7). The daily intramuscular administration of DFP to 60 normal subjects usually resulted in the development of symptoms referable to the central nervous system, including, in order of frequency, excessive dreaming, insomnia, jitteriness and restlessness, increased tension, emotional lability, subjective tremulousness, nightmares, headache, increased libido, giddiness, drowsiness, paresthesias, mental confusion, visual hallucinations, tremor, and pains in the legs of sciatic distribution. These symptoms were not significantly affected by the administration of neostigmine. They were diminished to some degree by the administration of atropine and by barbituric acid derivatives.

The production by DFP of symptoms which seemed to reflect increased neural activity suggested a study of the electroencephalographic changes that might follow the administration of this drug. This study was performed by means of a six channel Grass electroencephalograph employing both unipolar and bipolar leads, recordings being made before and during hyperventilation. Twenty-three subjects were observed, of whom nineteen had no demonstrable disease of the central nervous system or any neuromuscular abnormality, while four had myasthenia gravis, with normal electroencephalographic patterns.

[1] Work performed under a contract between the Medical Division, Chemical Corps, U. S. Army, and the Johns Hopkins University.

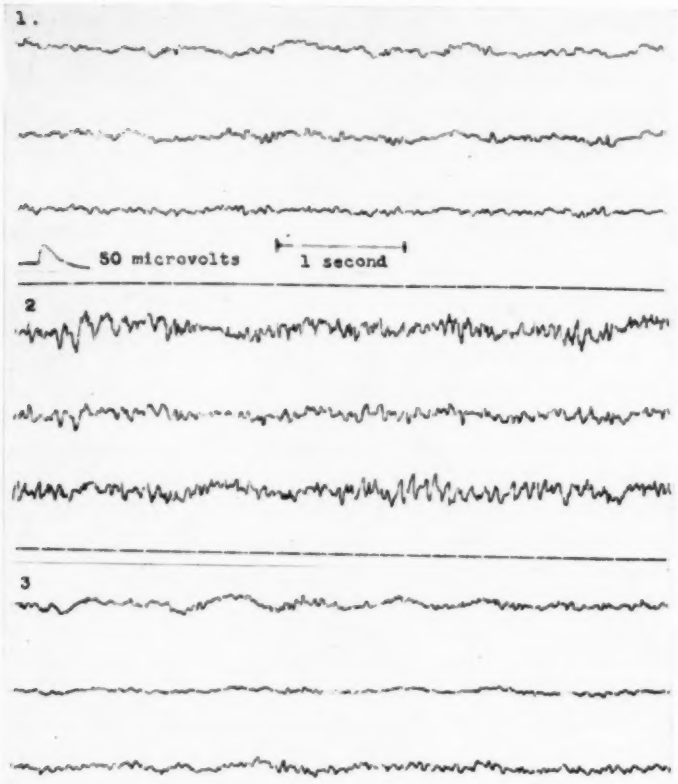

FIG. 1. Increased electrical activity of the brain following the administration of DFP to a normal subject (C. W.) and the antagonistic action of atropine. Unipolar leads: left frontal, central, and occipital leads recorded in that order. Amplification equal in all records. No hyperventilation. 1. Electroencephalogram before DFP. 2. Following the intramuscular administration of 1.5 mg. of DFP daily for three days. Cholinesterase 3 per cent (plasma) and 31 per cent (red blood cell) of original activity. 3. Five minutes after the intravenous administration of 1.2 mg. of atropine, and ten minutes after record 2.

THE EFFECT OF DFP ON THE ELECTROENCEPHALOGRAM

The administration of 1 to 2 mg. of DFP daily (intramuscularly) resulted in increased electrical activity of the brain in 17 of the 23 subjects (Figures 1 and 2). This was manifested by greater variations in

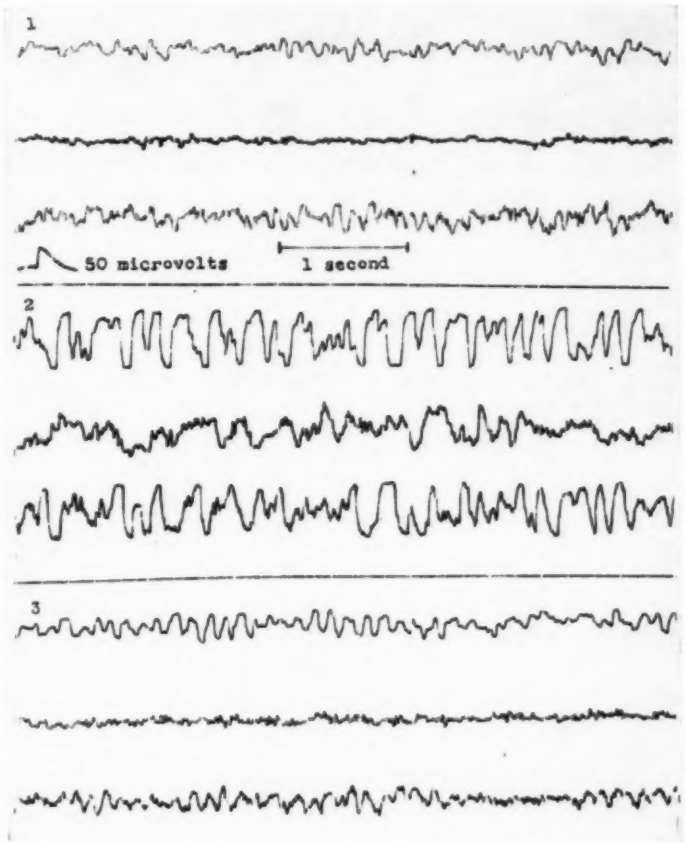

FIG. 2. Increased electrical activity of the brain following the administration of DFP to a normal subject (E. S.), and the antagonistic action of atropine. Bipolar leads: right central-frontal, frontal-frontal, and left central-frontal leads recorded in that order during the third minute of hyperventilation. Amplification equal in all records. 1. Electroencephalogram before DFP. 2. Following the intramuscular administration of 1.4 mg. of DFP daily for five days. Cholinesterase 12 per cent (plasma) and 30 per cent (red blood cell) of original activity. 3. Five minutes after the intravenous administration of 1.2 mg. of atropine, and ten minutes after record 2.

potential, by increased frequency (with increased beta rhythm), by more irregularities in rhythm, and by the intermittent appearance of

abnormal waves similar to those seen in patients with grand mal epilepsy. The latter consisted of slow waves (3 to 6 per second) of high voltage, usually most marked in the frontal leads, and increased by hyperventilation. In general, more striking electroencephalographic changes due to DFP occurred in those subjects who showed greater lability of pattern, though within the limits of normal, in their control records (Figure 2).

The electroencephalographic changes appeared after two to seven days of administration of DFP, and usually followed the onset of central nervous system symptoms. Following the cessation of DFP the symptoms due to this drug disappeared within one to four days (average two days), while the electroencephalographic changes persisted in diminishing degree for from 8 to 42 days (average 29 days).

CORRELATION WITH PLASMA AND RED BLOOD CELL CHOLINESTERASE

The onset or severity of central nervous system symptoms and electroencephalographic changes due to DFP bore no relation to the cholinesterase (ChE) activity of the plasma. When DFP was administered over a relatively short period of time (up to three days) the onset of symptoms and electroencephalographic changes could usually be correlated with the depression of red blood cell ChE to an average of 70 and 60 per cent of original activity, respectively. However, when DFP was administered over a longer period of time, or when administration of the drug was stopped, this correlation between central nervous system effects and red blood cell ChE activity no longer existed.

THE EFFECT OF ATROPINE ON CHANGES DUE TO DFP

The increased electrical activity of the brain due to DFP was inhibited in all of 17 subjects by the administration of atropine (Figures 1 and 2). The intravenous injection of 1.2 mg. of atropine to subjects who had received DFP resulted in an immediate decrease in potential and frequency (with decreased beta and increased alpha rhythm), a decrease in the irregularities of rhythm, and a decrease in the incidence of abnormal waves both before and during hyperventilation.

The daily administration of atropine, by any route, concomitant with the daily administration of DFP delayed the onset of both

central nervous system symptoms and electroencephalographic changes, the former for a few days and the latter for as long as three to four weeks, at which time the red blood cell ChE had been reduced to an average of 33 per cent of the original activity. Some of the subjects who received atropine and DFP daily for several weeks had pronounced central nervous system symptoms despite the absence of electroencephalographic changes. Withdrawal of atropine was followed within several days by the appearance of the electroencephalographic abnormalities.

THE EFFECT OF ATROPINE ON THE NORMAL ELECTROENCEPHALOGRAM

The administration of 1.2 mg. of atropine intravenously to 39 normal subjects resulted in some evidence of reduced electrical activity of the brain in over one-third of the subjects. In these cases there was an immediate decrease in voltage and frequency (with decreased beta and increased alpha rhythm), a decrease in the irregularities of rhythm, and a decrease in the appearance of abnormal slow waves during hyperventilation. Darrow (2) has reported previously that atropine produces changes in the normal electroencephalogram.

The administration of the same dose of atropine to 16 subjects with a history of grand mal epilepsy and with an electroencephalographic pattern characteristic of this disease resulted in some reduction of electrical activity and of abnormal waves in one-half of the subjects. These changes, which occurred immediately after the intravenous injection, were similar to those described as occurring in the normal subjects and in those who had received DFP, but were less marked than in the latter and were dramatic in only one case.

THE EFFECT OF NEOSTIGMINE AND CURARE ON CHANGES DUE TO DFP

The intravenous administration of 1.5 mg. of neostigmine, or 25 to 60 units of curare (Intocostrin, Squibb) or d-tubocurarine (Squibb) to 26 normal subjects, and to 13 subjects who had received DFP, resulted in no change in the normal electroencephalographic pattern, or in the central nervous system symptoms or electroencephalographic abnormalities due to DFP. The doses of curare and tubocurarine that were employed were sufficient to cause subjective weakness of the facial and ocular muscles, and with the highest dose objective weakness as well.

OTHER CENTRAL NERVOUS SYSTEM EFFECTS OF DFP

In addition to the symptoms and electroencephalographic changes which it produced, the administration of DFP resulted in other manifestations of increased central nervous system activity. These consisted of evidence of increased spinal cord activity and of spontaneous discharges within the spinal cord in two patients with upper motor neurone lesions due to late central nervous system syphilis. These patients had spastic paraplegia of the lower extremities, and no disturbance in the innervation of the upper extremities. The administration of DFP (2 mg. intramuscularly daily for three days) resulted in the familiar gastrointestinal, sudorific, and central nervous system symptoms. In addition to this, both patients developed intermittent and involuntary spontaneous clonic movements of the thigh and calf muscles which were increased greatly by passive stretching of the muscles and by voluntary movement. At the same time the weakness of the lower legs was increased and there was greater stiffness on walking. The spontaneous clonic movements, weakness, and stiffness were localized to the lower part of the body, and were only slightly inhibited by large doses of atropine. These symptoms subsided over a period of 72 hours after the last dose of DFP. They could not be reproduced by the administration of large doses of neostigmine (up to 200 mg. daily by mouth).

DISCUSSION

Mode of Action of DFP

The central nervous system symptoms, electroencephalographic changes, and evidence of spontaneous discharges within the spinal cord that resulted from the administration of DFP may be interpreted to result from the irreversible inhibition by DFP of ChE in the central nervous system. Measurement of this activity was not possible in man, but animal experiments have demonstrated the inactivation of brain ChE following the administration of DFP (8, 10). The persistence of the electroencephalographic changes due to DFP for as long as three and four weeks after the last dose of the drug probably reflects the slow regeneration of ChE in the central nervous system.

When the central nervous system symptoms and electroencephalo-

graphic changes due to DFP were evident there were no alterations in the respiratory rate, pulse rate, blood pressure, or carbon dioxide combining power of the serum to which these changes might be related.

Correlation of the Central Nervous System Effects of DFP with Plasma and Red Blood Cell Cholinesterase

The central nervous system effects of DFP bore no relation to the ChE activity of the plasma. They could be correlated with the ChE activity of the red blood cells only during the first three days of DFP administration, suggesting that the ChE enzymes of the central nervous system and red blood cells may have similar sensitivity to DFP. However, when DFP was administered over a longer period of time, or when the administration of the drug was stopped, this correlation no longer existed, presumably because of different rates of regeneration of the ChE enzymes in the central nervous system and in the red blood cells.

The Effect of Atropine, Curare, and Neostigmine on the Changes Due to DFP

Feldberg (3) has pointed out that, although there are many discordant findings, atropine is in general believed to inhibit the central nervous system effects of cholinergic drugs. The observed inhibitory action of atropine on the central nervous system symptoms and electroencephalographic abnormalities due to DFP is consonant with this view.

The persistence of the electroencephalographic changes due to DFP during sufficient curarisation to cause relaxation of the frontalis muscles makes it unlikely that the changes observed in the electroencephalogram were the result of spontaneous muscle activity underlying the electrodes. In addition, these observed changes did not resemble the effects of muscle activity.

The failure of neostigmine to duplicate or increase the central nervous system effects of DFP is in direct contrast to the effect of neostigmine on the gastrointestinal tract (7). This difference may be related to the lipoid solubility of neostigmine, which is much less than that of DFP. In view of the high lipoid content of the central nervous

system, the different effects of neostigmine and DFP might be due in part to the different solubilities of the two compounds, which would permit DFP to penetrate the central nervous system to a greater extent than neostigmine.

The Effect of an Upper Motor Neurone Lesion on the Action of DFP

The increased spinal cord activity and spontaneous discharges within the spinal cord which followed the administration of DFP to subjects with upper motor neurone lesions are compatible with the view that the central nervous system effects of DFP are mediated by increased cholinergic activity. Upper motor neurone section has been demonstrated to render the anterior horn cells (1), and the skeletal muscles supplied by such "denervated" anterior horn cells (9), more sensitive to stimulation by acetylcholine.

The Physiological Significance of the Central Nervous System Effects of DFP

A variety of experiments have been performed in an attempt to demonstrate the relationship of acetylcholine to the function of the central nervous system. These have been summarised in detail by Feldberg (3). The evidence is conflicting and the experimental procedures have usually been rather complicated. In the present study central nervous system effects have been produced by a compound, DFP, which to date, has been shown to have no other important action than its ability to destroy ChE. The production of these central nervous system effects by a compound which has been shown to inhibit ChE within the brain suggests that the acetylcholine cycle does play a positive, though as yet undefined, role in central neural function.

Compatible observations that have been reported in man include the occurrence of electroencephalic abnormalities following the intravenous administration of acetylcholine to epileptic subjects (11), and the occurrence of generalised convulsions following the intracisternal and intravenous administration of acetylcholine (4, 5).

SUMMARY

1. The administration of DFP, a potent anticholinesterase drug, to human subjects caused central nervous system symptoms and elec-

troencephalographic changes which indicated increased neural activity.

2. The electroencephalographic changes caused by DFP consisted of an increase in the potential, frequency, and irregularity of rhythm, and the intermittent appearance of abnormal waves similar to those seen in patients with grand mal epilepsy.

3. These electroencephalographic changes were promptly reversed by atropine and were not affected by neostigmine or curare. The central nervous system symptoms were inhibited to some degree by atropine.

4. Atropine caused some reduction in the electrical activity of the brain in one-third to one-half of the normal and epileptic subjects studied. The effect was similar to, but not as marked as, the effect of atropine on the electroencephalographic changes due to DFP.

5. The administration of DFP to two subjects with upper motor neurone lesions produced evidence of increased spinal cord activity and of spontaneous discharges within the spinal cord below the level of the lesions.

6. These central nervous system effects of DFP, their inhibition by atropine, and their increase in the presence of an upper motor neurone lesion suggest that the acetylcholine cycle plays a role in central neural function.

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THE ADMINISTRATION OF DI-ISOPROPYL FLUOROPHOSPHATE TO MAN

IV. The Effects on Neuromuscular Function in Normal Subjects and in Myasthenia Gravis[1]

A. M. HARVEY, J. L. LILIENTHAL, JR., D. GROB, B. F. JONES, AND S. A. TALBOT

From the Physiological Division, Department of Medicine, The Johns Hopkins University and Hospital, Baltimore, Maryland

The peculiar ability of di-isopropyl fluorophosphate (DFP) to inactivate cholinesterase (ChE) irreversibly provides a unique pharmacological tool for an analysis of certain cholinergic phenomena occurring during neuromuscular function, whether normal or disturbed. This paper reports the result of such a study in 15 normal subjects and in seven patients with myasthenia gravis. In addition, an assay has been made of the value of DFP in the treatment of myasthenia gravis in a group of ten patients with this disease in varying degrees of severity.[2]

METHODS

The injection of drugs under study followed the general plan described previously (9). Repeated injections were facilitated by introducing an inlying needle into the brachial artery. *DFP* was dissolved in physiological saline in a concentration of 1 mg. per ml. *Neostigmine* (Prostigmine, Hoffmann-LaRoche) was injected in an aqueous solution containing 0.5 mg. per ml. *Curare* (Intocostrin, Squibb) was injected in an aqueous solution containing 10 or 20 units per ml.

Grip strength was measured by a hand dynamometer (Stoelting). The electromyogram of the abductor of the 5th finger was recorded by the method of Harvey and Masland (7).

THE INTRA-ARTERIAL INJECTION OF DFP IN NORMAL SUBJECTS

DFP was injected into the brachial artery of 15 normal individuals in doses ranging from 0.5 to 2 mg. Measurable effects on neuromuscular conduction were observed after the smallest of these doses and increasing effects were noted following the larger doses. Atropine,

[1] Work performed under a contract between the Medical Division, Chemical Corps, U. S. Army, and the Johns Hopkins University.

[2] A preliminary abstract of this paper appeared in Fed. Proc., 1946, **5:** 182.

which was administered hypodermically prior to each injection in doses of 0.5 to 1.0 mg., did not influence the effect of DFP on neuromuscular conduction, and while it may have diminished sweating in the arm below the point of injection, in no case did it prevent this entirely.

Numerous spontaneous fasciculations appeared in the muscles of the injected area, having the same characteristics as those which follow the injection of neostigmine and those appearing in patients with

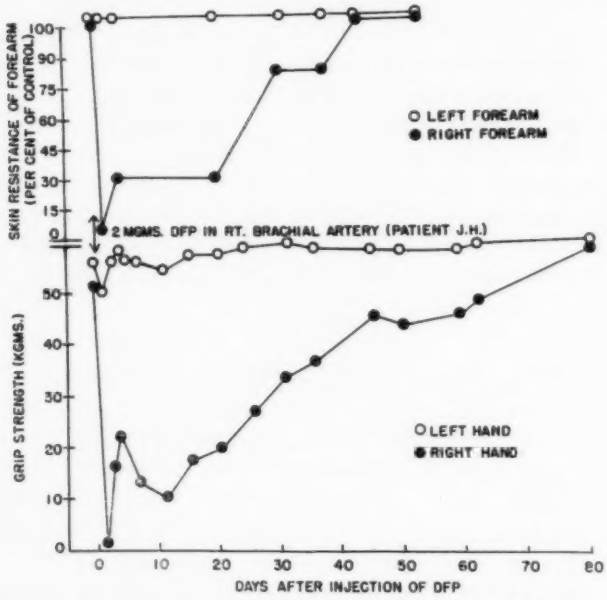

FIG. 1. The time course of the recovery in muscle power and skin resistance in a normal subject after the injection of 2 mg. of DFP into the right brachial artery.

chronic lower motor neurone disease. The subjects usually complained of coldness in the arm and hand below the point of injection.

Pronounced motor weakness was noted in the muscles of the injected area, its degree being proportional to the dosage of DFP. This weakness was not always uniform in distribution; for example, in one experiment the injection produced marked weakness of extension at the wrist and fingers which greatly overshadowed the weakness present in the flexor muscles in the forearm and hand. Figure 1 shows the

comparative strength of grip in the injected and uninjected hand after the administration of 2 mg. of DFP in the brachial artery. It may be noted that there was a very pronounced paresis of the muscles of the injected hand, and that strength returned slowly over a period of 11 weeks. This was accompanied by a decrease in skin resistance due to increased sweating with gradual return to normal over a period of six weeks. After a smaller dose of DFP (0.5 mg.) there was only slight reduction in strength and increase in sweating, and by the following day the only observable effect was easy fatigue of the muscles of the hand.

Electromyographic studies demonstrated the similarity of the effects of intra-arterial DFP and neostigmine on neuromuscular function. There was no change in the voltage of the muscle potential in response to a single maximal motor nerve stimulus. However, this response became repetitive in nature, and instead of the usual diphasic potential the initial spike was followed by a series of smaller potentials which showed progressive decline in voltage (Figures 2 and 3). Table 1 shows the voltage of the successive spikes and their time course. The response to a second stimulus, delivered within a period of 80 msec. after the first, did not show any evidence of the repetitive discharge. Other observations with various doses of DFP indicated that this period of suppression was proportional to the amount of repetition in the first response.

When two maximal nerve stimuli were delivered the voltage of the second muscle action potential was reduced. This reduction of voltage was greatest when the intervals between the two stimuli were short and lessened as the interval was increased. Table 2 shows the interval-potential relationship of this depression of the second of two responses at various time intervals after the intra-arterial injection of DFP. The voltage of the initial response is expressed as 100 per cent and the potential of the second response is recorded in terms of per cent of the first.

It is of interest that the maximal effect of the DFP was not reached immediately, but developed in 30 to 60 minutes. Except for the duration of the effect, which was found to be as long as 11 weeks in one patient, the changes observed in neuromuscular function were essentially similar to those observed after neostigmine where the maximal depression of function in the normal subject is seen within five minutes, and disappears within 20 to 45 minutes (9).

The changes seen in responses to repetitive nerve stimuli were also very striking. In Table 3 the successive responses are expressed in terms of per cent of the potential set up by the initial stimulus, the voltage of which is 100 per cent. The interval between the successive stimuli was 20 msec. This table demonstrates that after the injection

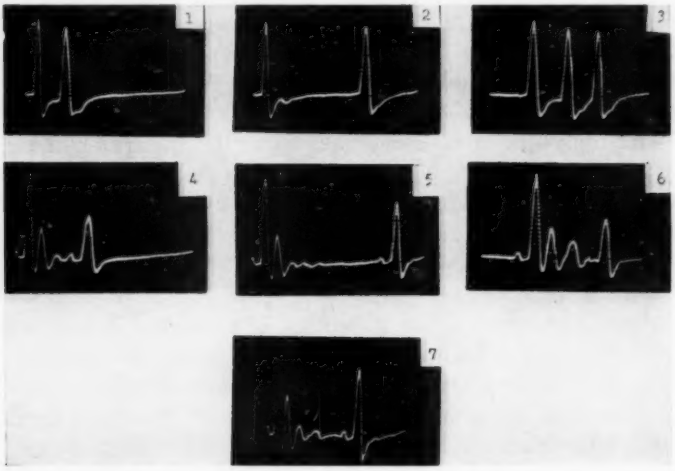

FIG. 2. Normal subject. Muscle action potentials in response to supramaximal motor nerve stimuli: *1 and 2:* Responses to 2 nerve stimuli at intervals of 30 and 80 msec. before DFP. *4 and 5:* Responses to 2 nerve stimuli 30 min. after the injection of 1.0 mg. of DFP into the brachial artery. *3 and 6:* Train of 3 responses before and after DFP (stimulation rate approx. 30 per sec.). *7:* Two responses after DFP at higher amplification to show the nature of the repetitive discharge more clearly. Voltage of the initial potential in (1) 8 mV. This figure illustrates the repetitive character of the initial response, the depressed voltage of the second response, and the varying responses to a train of stimuli, following the intra-arterial administration of DFP.

of DFP the second response is greatly depressed, but the third response shows a remarkable recovery. An example of this phenomenon is seen in Figure 3. This is in distinct contrast to the train of responses observed after the intra-arterial injection of neostigmine where there is a progressive decline in voltage of the successive potentials (Table 3). The significance of this difference in the effect of the two drugs on the

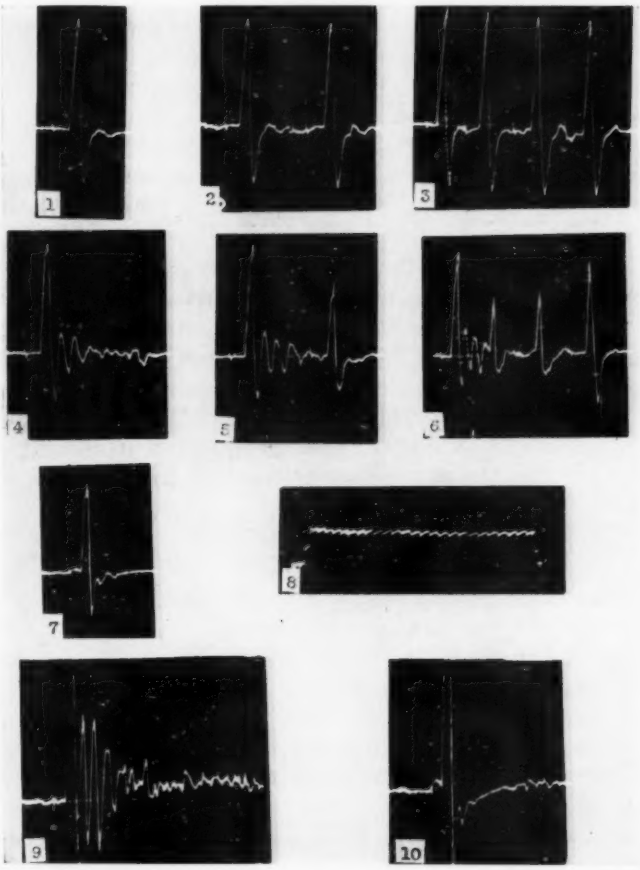

FIG. 3. The electromyogram of a normal subject before and after the intra-arterial injection of 0.5 mg. DFP and 9 units curare. *1, 2, and 3:* Control. *4, 5, and 6:* After DFP. *9:* Amplified record corresponding to 4 to show repetitive response. *8:* Calibration: 4mV. and 5 msec. *7:* After DFP followed by curare. *10:* Amplified record corresponding to 7 to show disappearance of repetitive response.

responses to a train of stimuli is not apparent from the observations made in the experiments described here.

In the normal subjects who received intra-arterial neostigmine a

TABLE 1

The muscle response to a single maximal motor nerve stimulus following the intra-arterial injection of DFP in a normal subject

	INITIAL SPIKE	REPETITIVE RESPONSES		
		1st	2nd	3rd
Voltage	6 mV.	3.4 mV.	1.8 mV.	0.52 mV.
Time of appearance	0	6.7 msec.	18 msec.	30 msec.

TABLE 2

The depression of the second of two responses of the muscle to maximal motor nerve stimuli in a normal subject

Figures represent voltage of second response expressed as per cent of initial response

INTERVAL BETWEEN STIMULI (MSEC.)	BEFORE DFP	TIME AFTER INJECTION OF DFP			
		5 min.	70–100 min.	20 hrs.	65 hrs.
10– 19	99	56		41	
20– 29			37	52	64
30– 39	97	66		74	76
40– 49	98	75	53	77	
50– 59					85
60– 69	97	88		82	
70– 79			66	84	85
80– 89		90			88
90– 99	98		69		
100–110			69		

TABLE 3

Muscle responses to repetitive maximal motor nerve stimuli at a rate of 50 per sec. in a normal subject

Figures represent per cent of voltage of response to initial stimulus

RESPONSE NO.	TIME AFTER DFP INJECTION (INTRA-ARTERIAL)					TIME AFTER NEOSTIGMINE INJECTION (INTRA-ARTERIAL)
	0	10 min.	70 min.	20 hrs.	65 hrs.	20 min.
1	100	100	100	100	100	100
2	97	50	32	24	46	74
3	97	59	45	58	74	45
4	96	72	53	66	59	26

spread of the effect throughout most of the muscles of the body was noted frequently within five minutes after release of the cuff, as indicated by the appearance of generalised fasciculations. No such generalised effects have been observed following even the largest doses of DFP injected intra-arterially. All of the manifestations have remained localised to the area distal to the site of intra-arterial injection.

Fasciculations were usually the first of the various neuromuscular manifestations to disappear. Thus, in the above patient who received 2 mg. of DFP, fasciculations were noted for only nine days. However, occasionally a few fasciculations have appeared following a dose of DFP which produced little or no motor weakness and in a few cases persisted after motor power had returned to normal. The repetitive response to a single stimulus usually was demonstrable for a longer period than the motor weakness. In one instance, there was motor weakness for only a few hours after the injection of 0.5 mg. of DFP while the repetitive discharge persisted for five days.

THE EFFECT OF CURARE UPON THE CHANGES PRODUCED BY THE INTRA-ARTERIAL INJECTION OF DFP IN NORMAL SUBJECTS

The effect of intra-arterial curare is demonstrated by the accompanying illustrations of the electromyograms of a normal male who received 0.5 mg. of DFP in the right brachial artery, and developed in the injected arm transient motor weakness, numerous fasciculations and diffuse sweating (Figure 3). The electromyographic changes which followed the injection of DFP were as described before: repetitiveness, depression of the second response to two stimuli, and the varying responses to a train of stimuli. Thirty-five minutes after the injection of DFP, nine units of curare were injected into the same artery. A bright flush appeared over the arm and there developed moderate, generalised swelling of the extremity with wheal formation (3). The repetitive response to single stimuli disappeared completely, and had not returned 30 minutes later. The voltage of the response to a single stimulus was reduced immediately following curare, but during the period of observation, slowly returned to the normal voltage. When virtually normal voltage had returned the repetitive response was still absent.

THE INTRA-ARTERIAL INJECTION OF DFP IN PATIENTS WITH MYASTHENIA GRAVIS

DFP has been injected intra-arterially in seven patients with myasthenia gravis on 14 occasions in doses ranging from 0.5 to 1.5 mg. The response was uniform in all of these patients and is well exemplified in the following experiment: After a period of 72 hours without neostigmine, the patient was given an injection of 0.5 mg. of DFP into the right brachial artery. Almost immediately profuse sweating appeared below the level of injection and local muscular power increased rapidly, reaching its maximum in approximately 15 minutes. No fasciculations were observed at any time following the injection. Electromyographic studies, made prior to the injection of DFP in this individual, showed the typical depression of the second of two action potentials in response to a pair of maximal motor nerve stimuli (8). When a train of four stimuli were applied to the nerve at intervals of 20 msec. there was a progressive decrease in size of muscle action potentials even during the period when the patient received 450 mg. of neostigmine orally each day. Table 4 shows the responses to such a train of stimuli during various stages of treatment. The character of the change in the electromyogram after the intra-arterial injection of DFP was similar to that which followed the injection of neostigmine (Figure 4). The improvement reached its maximum degree between one to two hours after the injection, but in this instance the dose apparently was insuficient to restore neuromuscular conduction to normal.

Table 5 shows the change in the successive responses to a train of stimuli at various time intervals after the intra-arterial injection of 0.5 mg. of DFP. Nine days after the injection of the DFP the dynamometer test showed persistent increased strength in the injected hand, 15 kg. as compared to a grip of 8 kg. before the injection. The control or uninjected hand had a grip strength of 8 kg. During this period there was persistent increase in sweating below the point of injection, and the patient stated that the hand on that side usually felt colder than the uninjected hand. It must be emphasised that at no time was there any increase in muscular strength elsewhere than distal to the level of intra-arterial injection. This is in contrast to the effect of neostigmine in myasthenia gravis where an intra-arterial injection of this drug produces a similar great increase in strength locally, but,

in addition, a generalised increase in motor power after the release of the cuff. On the third day after the injection of DFP the patient was

TABLE 4

Muscle responses to repetitive maximal motor nerve stimuli at a rate of 50 per sec. in a myasthenic subject during various stages of treatment

Figures represent voltage of response expressed as per cent of initial response

RESPONSE NUMBER	450 MG. NEOST. ORALLY IN 24 HRS.	OFF NEOST. 72 HRS.	15 MIN. AFTER 1.5 MG. NEOSTIG. INTRA-ART.
1	100	100	100
2	90	54	100
3	64	33	100
4	58	29	100

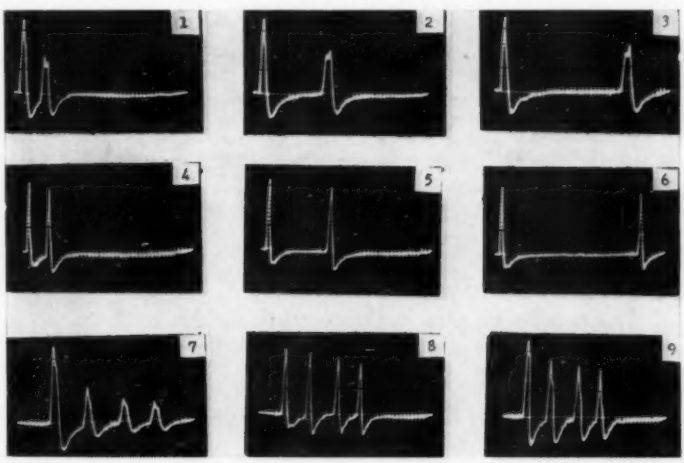

FIG. 4. Myasthenia gravis. Muscle action potentials in response to maximal motor nerve stimulation before and after the injection of 0.5 mg. DFP into the brachial artery. *1, 2, and 3:* Before DFP. Responses to two nerve stimuli at various time intervals. *4, 5, and 6:* Similar records 24 hours after DFP. *7:* Train of four stimuli (approx. 40 per sec.) before DFP. *8 and 9:* Similar trains at 24 and 48 hours after DFP.

given 1.5 mg. of neostigmine in the opposite (left) brachial artery to compare the maximum effects of the two drugs and to observe the time

relationship of the action of the two drugs when given by this route. Table 6 shows the grip strength of the two hands before and during this experiment. This maximum response reached by the right hand after

TABLE 5

Muscle responses to repetitive maximal motor nerve stimuli at a rate of 50 per sec. in a myasthenic subject before and at various time intervals after the intra-arterial injection of 0.5 mg. of DFP

Figures represent voltage of response expressed as per cent of initial response

RESPONSE	BEFORE DFP	15 MIN. AFTER	40 MIN.	4 HRS.	24 HRS.	96 HRS.
1	100	100	100	100	100	100
2	54	75	66	95	85	72
3	33	63	55	89	75	53
4	29	54	46	80	70	50

TABLE 6

The effect on the grip strength of a myasthenic subject of intra-arterial neostigmine administered 3 days after intra-arterial DFP

	GRIP STRENGTH (DYNAMOMETER)	
	Right hand (kg.)	Left hand (kg.)
(3 days after DFP (right artery))	18	8
Time after injection of 1.5 mg. Neostig. (left artery) (minutes)		
1	35	30
4	43	30
9	46	37
21	46	48
68	45	45
103	35	35
123	35	30
163	36	25
213	29	15
238	29	13
258	29	12
350	29	10

the injection of DFP was 44 kg. It is interesting that although the neostigmine was injected into the left brachial artery the muscles in the right arm, which had been injected previously with DFP, responded

more promptly and showed a more prolonged effect. However, the maximum degree of improvement was the same on the two sides.

One patient with extreme myasthenia gravis was studied with particular reference to the comparative effects of intra-arterial DFP and neostigmine. Before either drug was administered the voltage of the action potentials was low. After the intra-arterial injection of 1.5 mg. of neostigmine the voltage of the response to a single stimulus was doubled, and the responses to a train improved. The same degree of improvement was recorded on another occasion after the intra-arterial injection of 0.8 mg. of DFP.

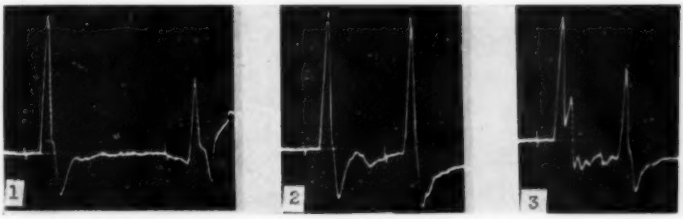

FIG. 5. Electromyogram of a myasthenic subject after DFP and after subsequent neostigmine. *1:* Control record; no medication for 7 hours. *2:* Repair of neuromuscular condition after 0.5 mg. of DFP intra-arterially. *3:* Deterioration of neuromuscular conduction following 0.5 mg. neostigmine (29 minutes after record 2) with development of repetitive discharge as seen in the first of the two responses.

THE INTERRELATIONSHIPS OF DFP AND NEOSTIGMINE

DFP Followed by Neostigmine

A patient with severe myasthenia gravis which involved the bulbar musculature predominantly, but with considerable weakness of the hands, received 0.5 mg. of DFP intra-arterially with subsequent improvement of neuromuscular conduction as measured by the response to a train of four stimuli. Nineteen minutes later he received 0.5 mg. of neostigmine in the same artery. Electromyographic measurements of neuromuscular conduction showed impairment, as evidenced by a depression in the voltage of the second response to a pair of stimuli and by the appearance of repetitive response to the first stimulus (Figure 5).

Thus, in a muscle exhibiting a typical myasthenic response, neuro-

muscular conduction was repaired by intra-arterial DFP; a subsequent injection of neostigmine then depressed neuromuscular conduction and induced repetitive discharge in a fashion similar to its effect on normal muscle. This train of events, which represents the first observation of this nature in our experience, might be interpreted to result from the additive anti-cholinesterase effects of an initial dose of DFP followed by a subsequent dose of neostigmine which permitted the development of a paralysing concentration of acetylcholine at the neuromuscular junction. This indicates that neostigmine administered after DFP can produce a summated anti-cholinesterase effect.

Neostigmine Followed by DFP

In the experiments in which DFP was injected intra-arterially it was noted that the effect was appreciably less in patients who had received neostigmine shortly before the injection of the DFP. Also, it was observed that when DFP was given intramuscularly to a myasthenic patient who was receiving his regular neostigmine medication the effect of the DFP was reduced. These observations suggested that neostigmine inhibited the effect of DFP. To analyse this relationship in more detail, two patients with myasthenia gravis were given on five occasions an injection of DFP into the brachial artery at a time when they had received no neostigmine during the preceding 24 hours. Injections of DFP were repeated after an interval of one week in the opposite brachial artery at a moment when the patients were at the point of maximal benefit from an intramuscular injection of 2 mg. of neostigmine. Figure 6 demonstrates that when the patient had had a recent injection of neostigmine the intra-arterial injection of DFP did not produce the lasting increase in muscular power which followed the administration of DFP alone. The results were uniform in all of these experiments. These observations suggest that neostigmine and DFP compete for a common site of action, and that when neostigmine is present at this site it blocks the action of DFP. In order to exclude the possibility that this effect may have been due to the residual presence of DFP the blocking experiment was done on several occasions in patients who had never received a previous injection of DFP. The results were entirely similar to those in which the patient had received a DFP injection one week prior to the blocking experiment. Addi-

tional observations showed that the presence of DFP does not block the action of subsequent injections of this drug; in one patient DFP injections were made into the same artery at an interval of three days and the second injection produced the same maximum response as that which had resulted originally.

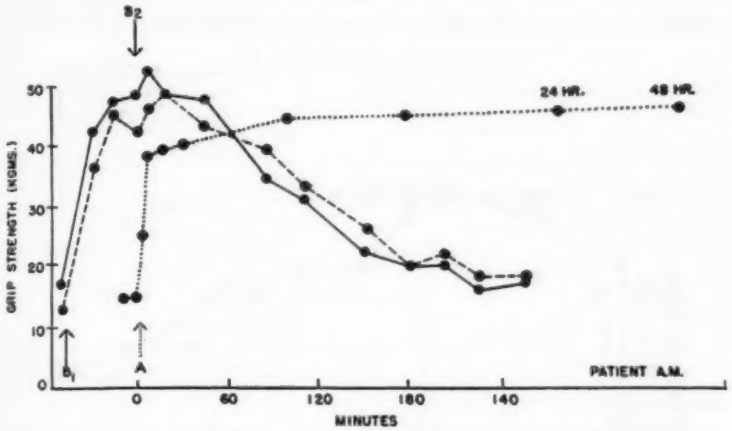

FIG. 6. The blocking effect of neostigmine on the response of the myasthenic muscle to DFP injected intra-arterially. . . . . Right grip showing the prolonged response of the muscles to DFP injected into the right brachial artery at A when the patient had had no neostigmine for 24 hours. ———— Left grip, - - - - Right grip in an experiment done one week later showing the response to 2 mg. of neostigmine administered intramuscularly at B^1. At B^2 0.65 mg. of DFP was injected into the left brachial artery while the neostigmine effect was at its height. The strength in the two hands returned to their original levels at the same time, in contrast to the prolonged effect of DFP in the absence of neostigmine.

THE TREATMENT OF PATIENTS WITH MYASTHENIA GRAVIS WITH DFP

Ten patients with myasthenia gravis have been treated for periods of time varying from a few weeks to several months with intramuscular injections of DFP in oil. These patients were selected to represent most of the variations observed in the clinical picture of myasthenia gravis, with cases ranging from early and very mild forms of the disease to patients in whom the disease had been present a long time and was

very severe. As shown in Table 7 the distribution in some instances was primarily bulbar while in others the muscles of the trunk and extremities were predominantly involved. The cases also presented a fairly inclusive picture in relation to previous treatment. One individual had never before received any drug for the treatment of myasthenia gravis while others had received neostigmine for a short period of time and, in many, treatment with this drug had been con-

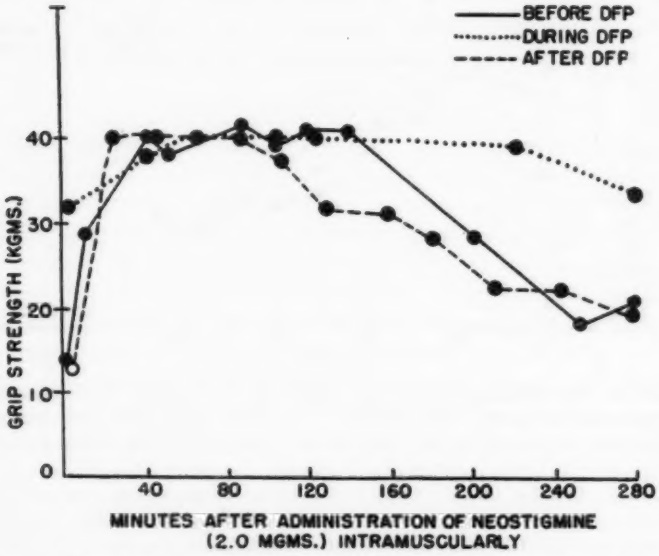

FIG. 7. The effect of neostigmine in a patient with myasthenia gravis before, during, and after the administration of DFP. Although the basal strength was greater during DFP therapy, the maximum response to the neostigmine was the same.

stant over a number of years. In four individuals thymectomy had been performed. In two of these this was done before DFP treatment was given, but in two others the drug was administered both before and after the removal of the thymus gland. Because of the recognised emotional states which may occasionally simulate this disease, great care was taken to establish the diagnosis beyond question in each case. A variety of facts were gathered for this purpose. Each patient was

examined by several physicians, and agreement as to the diagnosis was uniform. A variety of other examinations were carried out to substantiate the diagnosis, including therapeutic tests with neostigmine, administration of quinine, intra-arterial injection of neostigmine, and detailed electromyographic studies following supra-maximal stimulation of the motor nerve. No individual was included in this study unless these various tests and examinations gave incontrovertible

TABLE 7

Description of cases of myasthenia gravis treated with DFP

SUBJECT	AGE	SEX	COLOR	DISTRIBUTION OF WEAKNESS		SEVERITY	DURATION (YEARS)	HISTORY OF REMISSION	TREATMENT WITH NEOSTIGMINE (MG.)		MYOTONIA	REMARKS
				Oculobulbar	Peripheral				Oral	Hypodermal		
B. S.	23	F	W	+++	+++	+++	14	0	360	1.0*	0	
A. M.	30	M	C	+	++++	+++	1	0	270	1.0*	+	Thymectomy after treatment with DFP
J. H.	23	M	W	++++	++	+++	1	0	270	4.0	0	
R. N.	30	F	W	++++	++	+++	5	0	225	2.0	0	
R. P.	34	M	C	++++	++	+++	12	Partial after thymectomy	150	1.0*	+	Thymectomy before treatment with DFP
B. W.	18	F	C	+++	++	+++	6	Partial after thymectomy	150	1.0*	+	Thymectomy before treatment with DFP
J. W.	30	F	W	+++	++	+++	1½	0	120	0	0	
L. C.	24	F	W	0	+++	++	7	0	90	0	0	
F. T.	10	F	C	++	+	++	1	0	90	0.5*	0	No treatment with neostigmine before trial on DFP
C. P.	41	M	W	++++	++++	++++	1	0	0	20	+	Thymectomy after treatment with DFP

* Rarely.

evidence that the patient had myasthenia gravis. DFP was administered in doses ranging from 0.5 to 2 mg., given intramuscularly in peanut oil, and repeated usually at daily intervals until the cholinesterase had been reduced to the maximum degree possible without the production of unbearable symptoms. When sufficient doses of atropine were given to control symptoms it was possible to reduce the red cell esterase to very low levels. The autonomic and central neural

symptoms which these patients developed as a result of DFP administration were essentially the same as those observed in normal individuals (4). Atropine did not alter the effects of DFP on skeletal muscle.

In general, DFP has not proved to be a promising therapeutic agent for myasthenia gravis. A definite beneficial effect was obtained in each of the ten individuals, but only in the cases with very mild weakness were the patients able to carry on any degree of activity approaching that possible with neostigmine. Symptoms due to the reduction of cholinesterase activity in the central nervous system and gastro-intestinal tract were almost uniformly present in these individuals and with one exception the patients much preferred treatment with neostigmine. In the severe cases, DFP was noted to have a slight effect in enhancing the response to neostigmine, but it was not possible to bring about more than a moderate reduction in neostigmine requirement in cases of this type without doses of DFP which caused undesirable central nervous system and gastro-intestinal symptoms. In the mild and moderately severe cases it was possible to reduce the daily neostigmine requirement to a significant degree. In these patients it was observed that when they were receiving DFP they could reach their maximum possible response to neostigmine with smaller doses of the latter drug. All of the myasthenic patients, after a rest period overnight without medication and tested in a basal state in the morning, were observed to have an increase in their strength and in the variety of activities which they could carry out without medication. However, these improvements in their clinical condition did not usually approach that possible with satisfactory amounts of neostigmine, and it was found that when neostigmine was given in addition to the basal dose of DFP the maximum increase in strength never exceeded that which was possible on neostigmine alone (Figure 7 and Table 8).

Additional evidence of this limitation of the physiological capacity of neuromuscular function was indicated by the fact that with DFP some of these patients could be brought to a maximum degree of strength, beyond which an added dose of neostigmine carried them over into a state of weakness simulating that seen in normal individuals following an excessive dose of an anticholinesterase drug (Figure 5).

In an assessment of the value of DFP it was found that this drug had to be given at a time when the patient was not under the influence

TABLE 8

The influence of the daily administration of DFP on the cholinesterase activity (plasma and red blood cell), neostigmine requirement, and strength of 10 myasthenic patients

PATIENT	NO. OF DAYS	DFP AV. DAILY DOSE (MG.)	BASAL CHOLINESTERASE AT END OF EACH PERIOD (%)		NEOSTIGMINE AV. DAILY REQUIREMENT (MG. ORAL)	GRIP STRENGTH (KG.)		BASAL CONDITION
			Plasma	RBC		Av. basal last 5 days of each period	Max. after neostigmine	
B. S.	12	0	100	100	360	8	33	Unable to get out of bed or swallow food.
	37	0.44	9	23	219	21	34	General strength moderately improved; could do above.
	10	0	52	32	261	21	35	Returned to original condition within two weeks.
A. M.	22	0	100	100	270	13	52	Unable to lift head off pillow or roll over in bed.
	10	1.00	10	42	180	28	54	General strength moderately improved; could do above.
	10	0	58	52	204	13	54	Returned to original condition within one week.
J. H.	7	0	100	100	261	15	48	Unable to get out of bed or swallow liquid.
	33	1.04	10	22	109	30	51	General strength moderately improved; could do above.
	15	0	85	31	137	17	42	Returned to original condition in two weeks.
R. N.	37	0	100	100	223	8	29	Unable to get out of bed or swallow liquid.
	25	1.25	10	22	106	15	30	General strength moderately improved; could do above.
	17	0	79	34	129	9	30	Returned to original condition within two weeks.

TABLE 8—*Continued*

PATIENT	NO. OF DAYS	DFP AV. DAILY DOSE (MG.)	BASAL CHOLINESTERASE AT END OF EACH PERIOD (%)		NEOSTIGMINE AV. DAILY REQUIREMENT (MG. ORAL)	GRIP STRENGTH (KG.)		BASAL CONDITION
			Plasma	RBC		Av. basal last 5 days of each period	Max. after neostigmine	
R. P.	11	0	100	100	131	36	66	Ptosis, diplopia, facial weakness, difficulty swallowing, easy fatigue.
	64	0.69	7	3	0	46	52	General strength and above symptoms moderately improved.
	34	0	85	30	36	34	52	Returned to original conditions within three weeks.
B. W.	16	0	100	100	124	8	25	Unable to get out of bed; ptosis, facial weakness; difficulty swallowing.
	56	0.66	8	48	89/0	30	32	General strength and above symptoms markedly improved.
	8	0						On eighth day died outside of hospital in sudden exacerbation of weakness.
J. W.	29	0	100	100	119	24	39	Ptosis, diplopia, facial weakness, unable to swallow food.
	33	1.13	6	15	92	34	40	General strength and above symptoms moderately improved.
	21	0	77	41	91	28	42	Strength improved over original condition after four weeks.

TABLE 8—*Concluded*

PATIENT	NO. OF DAYS	DFP AV. DAILY DOSE (MG.)	BASAL CHOLINESTERASE AT END OF EACH PERIOD (%)		NEOSTIGMINE AV. DAILY REQUIREMENT (MG. ORAL)	GRIP STRENGTH (KG.)		BASAL CONDITION
			Plasma	RBC		Av. basal last 5 days of each period	Max. after neostigmine	
L. C.	15	0	100	100	69	15	39	Unable to get out of bed.
	8	1.69	9	46	49	30	40	General strength slightly improved; could do above.
	20	0	64	54	67	16	40	Returned to original condition within one week.
F. T.	11	0	100	100	0	12		Ptosis, diplopia, easy fatigue.
	11	0.42	21	17	0	20	22	General strength and above symptoms moderately improved.
	11	0	46	36	0	16	23	Returned to original condition within one week.
C. P.	18	0	100	100	20 (mg. hypo)	0	22	Unable to lift head off pillow or swallow liquid; ptosis, diplopia.
	27	0.46	13	41	17 (mg. hypo)	3	15	General strength and above symptoms slightly improved.
	8	0	37	51	20 (mg. hypo)	0		Returned to original condition within one week.

of neostigmine; it was administered, therefore, early in the morning after the patient had passed the night without medication. In this way the possible competition of these two drugs for a similar site of action could be excluded as a factor affecting the clinical response to DFP.

All of these patients were followed carefully after the cessation of DFP treatment and in most of them there was a gradual decline in basal muscular strength, the details of which can be seen in Table 8. Plasma and red blood cell cholinesterase activity rose gradually to the previous normal levels. The daily neostigmine requirement which was reduced during the period of DFP treatment returned gradually to its previous level. There was no demonstrable difference in the response to DFP before and after thymectomy.

It is noteworthy that following cessation of DFP treatment three of the patients in this series died, all with essentially the same clinical picture. In each instance the patient developed an infection of the upper respiratory tract which was followed by rapid increase in weakness, acute respiratory distress and death in a few hours. The first patient (B. W.) had received 37 mg. of DFP in 56 days, had had no DFP for a period of eight days, had complained of no symptoms which could be attributable to the anticholinesterase activity of this drug, and had been visiting the hospital daily for check-up. The morning of her death, which was the ninth day after the last dose of DFP, she arose, took 30 mg. of neostigmine by mouth and started for the hospital in apparently good health except for a mild cold. While on the way she suddenly became extremely short of breath, and was immediately sent to the hospital where she arrived unconscious. Efforts to revive her failed, and she died within a few hours.

The second patient (R. P.) had a rather severe form of the disease which had been characterised over a period of years by numerous spontaneous remissions and relapses following infections of the upper respiratory tract. He was treated for a period of two months with DFP (total dose 44 mg.) and was in the hospital for observation after the cessation of this drug during the period of readjustment to neostigmine. He remained under observation for eight weeks during which time he showed wide variations in strength and in neostigmine requirements. During this period he contracted an upper respiratory tract infection which made difficult the control of his myasthenia gravis. He was discharged from the hospital at his own request 55 days after the last dose of DFP on a daily neostigmine dosage of 150 mg. by mouth, amplified by occasional hypodermic injections of 1 mg. for nocturnal choking spells. During the following ten days his strength

declined, and he required frequent emergency doses of neostigmine. He was urged to return to the hospital, but refused. On the ninth day after discharge and the 64th day after the last dose of DFP, he developed a sudden exacerbation of his weakness with choking, and died on the way to the hospital.[8]

The third patient (J. W.) was a 30 year old married female who received DFP in oil for 33 days (in doses varying from 1 to 1.5 mg.) for a total of 37 mg. She had a good response to this drug, with a moderate increase in basal strength, and some decrease in neostigmine requirement. She had no other symptoms attributable to the DFP. She was discharged from the hospital 34 days after her last dose of DFP, at which time she had undergone what was considered a slight remission of her disease, as her basal strength was somewhat improved. Almost five months after her last dose of DFP she developed a mild infection of the upper respiratory tract, became rapidly weaker, and died of respiratory paralysis before she could be brought to the hospital.

DISCUSSION

Comparison of Action of DFP and Neostigmine

DFP and neostigmine both inhibit ChE, the former irreversibly, the latter reversibly. This difference in their actions becomes apparent when the duration of effects following intra-arterial injection are compared. The local sweating, weakness and fasciculations disappear within one hour after neostigmine; after DFP these effects may persist for hours, days or weeks depending upon the amount injected. The rate of return of normal function probably reflects the rate of local regeneration of ChE.

The injection of either DFP or neostigmine converts the single diphasic electrical response of normal muscle to a single shock stimulation of the motor nerve into a repetitive response. This evidence of hyperexcitability of the neuromuscular unit is observed only after the first stimulus; a second stimulus presented within less than 110 msec. evokes a reduced response which is not repetitive. This sequence of hyperexcitability and depression is consonant with the common anti-

[8] Autopsies performed on patients B. W. and R. P. revealed lobular pneumonia sufficient in extent to explain sudden exitus in a patient with myasthenia gravis (4).

cholinesterase action of these agents, and has been interpreted to indicate, first, persistence at the nerve endings of acetylcholine and, then, accumulation of acetylcholine to paralysing concentrations (9).

Eccles, Katz and Kuffler (2) in a study of the effect of another anticholinesterase agent, eserine, on the myoneural junction, attributed the repetitive discharge and neuromuscular block to the prolongation of the endplate potential which eserine produces. However, this interpretation of the events occurring at the neuromuscular junction after treatment with eserine could not be applied without modification to the events occurring after injection of DFP. In Figures 2 and 3 it will be noted that the second response to a train exhibits the greatest depression and that although the subsequent responses do not become repetitive they do regain some lost potential. This demonstration of recovery of neuromuscular transmission during a train of stimuli cannot be explained adequately at present.

Another difference between the actions of DFP and neostigmine injected intra-arterially was noted in the consistent localisation of effects to the injected arm after DFP in contrast to the generalised effects which followed neostigmine. This difference was especially marked in patients with myasthenia gravis where DFP produced a local increase in strength in the injected arm but virtually none elsewhere, in contrast to the effects of neostigmine which are generalised regardless of the route of administration. The available evidence furnishes no adequate explanation for this phenomenon, although known differences in the kinetics of reaction of these agents with cholinesterases, and in their solubilities, may play important rôles.

The blocking of DFP effect by prior administration of neostigmine may well be the result of different modes of reaction of these agents with ChE. It may be assumed that after the intra-arterial injection of neostigmine a major portion of the ChE in the arm is combined with neostigmine, and that this ChE-neostigmine complex is not susceptible to attack by the subsequently injected DFP which is then destroyed by fluorophosphatase. Then, presumably, in the course of the next hour, the ChE is released from its combination with neostigmine into an environment which has been freed from DFP. This phenomenon has been noted in vitro (10), and in vivo in the blocking of DFP action

on the gut (5) and by the protective action of physostigmine against the toxic effects of DFP in cats (11).

If these drugs are injected in the reverse order, DFP followed by neostigmine, the effects are summed rather than opposed. In this case it is probable that the remaining ChE which has not been destroyed by DFP is inhibited by neostigmine. In the example of this additive effect which has been described above a chance combination of doses of these agents evoked in a patient with severe myasthenia gravis a paralysing response to neostigmine. The sequence of events suggests that the combined effects permitted a paralysing concentration of acetylcholine to accumulate at the neuromuscular junction in an instance of myasthenia gravis. This "normal" response to neostigmine was only partial, however, because spontaneous fasciculations did not occur.

The characterisation of phenomena occurring at the neuromuscular junction in terms of known acetylcholine-cholinesterase relationships has proved extremely useful in many attempts to interpret the effects of drugs and disease. Hitherto these interpretations have been based on the assumption that the effects of neostigmine were limited to an inhibition of ChE. The recent report of a direct action of neostigmine on skeletal muscle may require that these earlier interpretations be modified (12).

Curare injected after the DFP effects were well established produced some noteworthy changes. First, there appeared a transient increase in the weakness established by DFP. If the effects of DFP and curare on the neuromuscular junction were the result simply of an excess of acetylcholine (DFP) or lessened response of muscle to acetylcholine (curare) then it might be anticipated that curare would lessen the weakness produced by DFP. This antagonism has been demonstrated and measured experimentally for eserine, another anticholinesterase agent (2). It may well be that the failure of curare to lessen the weakness following DFP in this study was the result of comparative dosage levels; i.e., the amount of curare injected was sufficient to produce neuromuscular block by increasing muscle threshold to acetylcholine to such a degree that any DFP-curare antagonism was masked. A second possibility to be considered is that the preparation of curare

(Intocostrin) used in this experiment may contain impurities which are themselves anticholinesterase in action (6).

That curare did antagonise certain effects of DFP was demonstrated by the suppression of repetitive responses to single stimuli which continued after neuromuscular transmission had returned to normal levels. The counterpart of this effect has been analysed in eserinised experimental animals by Eccles, Katz and Kuffler and explained by their observation that curare reverses the eserine effect of prolonging the endplate potential (2).

The Treatment of Myasthenia Gravis with DFP

The experience in treating myasthenia gravis with DFP has been discouraging in general (1). It is true that definite improvement was observed in several instances but in none was there noted a beneficial effect equal to that afforded by neostigmine. Furthermore the development of unpleasant central neural and gastro-intestinal side-effects precluded vigorous treatment with DFP. When these central neural effects were well established they were not accentuated by the administration of neostigmine, suggesting that their appearance was related to the high lipoid solubility of DFP which permitted its penetration into the neuraxis, in contrast to neostigmine which is less soluble in lipoids. From these considerations it would seem likely that the general treatment of myasthenia gravis with anticholinesterase agents of this type must await the development of a substance which, by reason of its distribution within the body, will produce maximal effects in striated muscle before it destroys appreciable amounts of ChE in the central nervous system.

We are indebted to Dr. John R. Brewer of Hynson, Westcott, and Dunning, Baltimore, Maryland for his help in ampouling our solutions of DFP.

SUMMARY

1. In normal subjects intra-arterial injections of DFP produced localised effects distal to the site of injection which were similar in most respects to those produced by neostigmine: weakness, fasciculations, sweating, electromyographic evidence of repetitive response and

depression of neuromuscular transmission. DFP differed from neostigmine in producing its effects more slowly, in the prolonged duration of effects, in the varying responses to a train of stimuli, and in the absence of generalised action.

2. In patients with myasthenia gravis intra-arterial injection of DFP produced local return of motor power which persisted for prolonged periods.

3. Evidence has been adduced to indicate that DFP and neostigmine compete for ChE, and that the formation of a neostigmine-ChE complex will protect the ChE from irreversible inhibition by subsequently injected DFP, thereby blocking the effect of DFP.

4. Treatment of ten patients with myasthenia gravis by DFP resulted in appreciable gain in strength but in no instance was the improvement as great as that obtained with neostigmine. The unpleasant central nervous and gastro-intestinal symptoms produced by DFP precluded its use in amounts sufficient to produce adequate therapeutic effects.

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